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(54) Title: DETERMINATION OF CANCEROUS CONDITIONS BY MAGE GENE EXPRESSION (57) Abstract A method for determining cancers is described. The method involves assaying for expression of a gene coding for at least one of MAGE tumor rejection antigen or its precursor expression product.		

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DETERMINATION OF CANCEROUS CONDITIONS BY MAGE GENE EXPRESSION

FIELD OF THE INVENTION

5 This invention relates to general methods for
diagnosing cancers via determining expression of at least
one member of the MAGE family of tumor rejection antigen
precursors. More particularly, cancers such as lung
10 cancers can be diagnosed by determining expression of one
or more members of this family of genes. Also a part of
the invention are primers which can be used in these
methods, such as amplification methods, of which the
polymerase chain reaction ("PCR") is the most well known.

BACKGROUND AND PRIOR ART

15 The study of the recognition or lack of recognition of
cancer cells by a host organism has proceeded in many
different directions. Understanding of the field presumes
some understanding of both basic immunology and oncology.
20 Early research on mouse tumors revealed that these
displayed molecules which led to rejection of tumor cells
when transplanted into syngeneic animals. These
molecules are "recognized" by T-cells in the recipient
animal, and provoke a cytolytic T-cell response with lysis
25 of the transplanted cells. This evidence was first
obtained with tumors induced in vitro by chemical
carcinogens, such as methylcholanthrene. The antigens
expressed by the tumors and which elicited the T-cell
response were found to be different for each tumor. See
30 Prehn, et al., J. Natl. Canc. Inst. 18: 769-778 (1957);
Klein et al., Cancer Res. 20: 1561-1572 (1960); Gross,
Cancer Res. 3: 326-333 (1943), Basombrio, Cancer Res. 30:
2458-2462 (1970) for general teachings on inducing tumors
with chemical carcinogens and differences in cell surface
35 antigens. This class of antigens has come to be known as

"tumor specific transplantation antigens" or "TSTAs". Following the observation of the presentation of such antigens when induced by chemical carcinogens, similar results were obtained when tumors were induced in vitro via
5 ultraviolet radiation. See Kripke, J. Natl. Canc. Inst. 53: 333-1336 (1974).

While T-cell mediated immune responses were observed for the types of tumor described supra, spontaneous tumors were thought to be generally non-immunogenic. These were
10 therefore believed not to present antigens which provoked a response to the tumor in the tumor carrying subject. See Hewitt, et al., Brit. J. Cancer 33: 241-259 (1976).

The family of tum antigen presenting cell lines are immunogenic variants obtained by mutagenesis of mouse tumor
15 cells or cell lines, as described by Boon et al., J. Exp. Med. 152: 1184-1193 (1980), the disclosure of which is incorporated by reference. To elaborate, tum antigens are obtained by mutating tumor cells which do not generate an immune response in syngeneic mice and will form tumors
20 (i.e., "tum⁺" cells). When these tum⁺ cells are mutagenized, they are rejected by syngeneic mice, and fail to form tumors (thus "tum⁻"). See Boon et al., Proc. Natl. Acad. Sci. USA 74: 272 (1977), the disclosure of which is incorporated by reference. Many tumor types have been
25 shown to exhibit this phenomenon. See, e.g., Frost et al., Cancer Res. 43: 125 (1983).

It appears that tum variants fail to form progressive tumors because they initiate an immune rejection process. The evidence in favor of this hypothesis includes the
30 ability of "tum⁻" variants of tumors, i.e., those which do not normally form tumors, to do so in mice with immune systems suppressed by sublethal irradiation, Van Pel et al., Proc. Natl. Acad. Sci. USA 76: 5282-5285 (1979); and the observation that intraperitoneally injected tum cells
35 of mastocytoma P815 multiply exponentially for 12-15 days, and then are eliminated in only a few days in the midst of

an influx of lymphocytes and macrophages (Uyttenhove et al., J. Exp. Med. 152: 1175-1183 (1980)). Further evidence includes the observation that mice acquire an immune memory which permits them to resist subsequent challenge to the same tum variant, even when immunosuppressive amounts of radiation are administered with the following challenge of cells (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977); Van Pel et al., supra; Uyttenhove et al., supra).

Later research found that when spontaneous tumors were subjected to mutagenesis, immunogenic variants were produced which did generate a response. Indeed, these variants were able to elicit an immune protective response against the original tumor. See Van Pel et al., J. Exp. Med. 157: 1992-2001 (1983). Thus, it has been shown that it is possible to elicit presentation of a so-called "tumor rejection antigen" in a tumor which is a target for a syngeneic rejection response. Similar results have been obtained when foreign genes have been transfected into spontaneous tumors. See Fearon et al., Cancer Res. 48: 2975-1980 (1988) in this regard.

A class of antigens has been recognized which are presented on the surface of tumor cells and are recognized by cytolytic T cells, leading to lysis. This class of antigens will be referred to as "tumor rejection antigens" or "TRAs" hereafter. TRAs may or may not elicit antibody responses. The extent to which these antigens have been studied, has been via cytolytic T cell characterization studies, in vitro i.e., the study of the identification of the antigen by a particular cytolytic T cell ("CTL" hereafter) subset. The subset proliferates upon recognition of the presented tumor rejection antigen, and the cells presenting the antigen are lysed. Characterization studies have identified CTL clones which specifically lyse cells expressing the antigens. Examples of this work may be found in Levy et al., Adv. Cancer Res. 24: 1-59 (1977); Boon et al., J. Exp. Med. 152: 1184-1193 (1980); Brunner et al., J. Immunol. 124: 1627-1634 (1980);

Maryanski et al., Eur. J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982); Palladino et al., Canc. Res. 47: 5074-5079 (1987). This type of analysis is required for other types of antigens recognized by CTLs, including minor histocompatibility antigens, the male specific H-Y antigens, and the class of antigens referred to as "tum-" antigens, and discussed herein.

A tumor exemplary of the subject matter described supra is known as P815. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988); Szikora et al., EMBO J 9: 1041-1050 (1990), and Sibille et al., J. Exp. Med. 172: 35-45 (1990), the disclosures of which are incorporated by reference. The P815 tumor is a mastocytoma, induced in a DBA/2 mouse with methylcholanthrene and cultured as both an in vitro tumor and a cell line. The P815 line has generated many tum variants following mutagenesis, including variants referred to as P91A (DePlaen, supra), 35B (Szikora, supra), and P198 (Sibille, supra). In contrast to tumor rejection antigens - and this is a key distinction - the tum antigens are only present after the tumor cells are mutagenized. Tumor rejection antigens are present on cells of a given tumor without mutagenesis. Hence, with reference to the literature, a cell line can be tum⁺, such as the line referred to as "P1", and can be provoked to produce tum variants. Since the tum phenotype differs from that of the parent cell line, one expects a difference in the DNA of tum cell lines as compared to their tum⁺ parental lines, and this difference can be exploited to locate the gene of interest in tum cells. As a result, it was found that genes of tum variants such as P91A, 35B and P198 differ from their normal alleles by point mutations in the coding regions of the gene. See Szikora and Sibille, supra, and Lurquin et al., Cell 58: 293-303 (1989). This has proved not to be the case with the TRAs of this invention. These

papers also demonstrated that peptides derived from the tumor antigen are presented by the L^d molecule for recognition by CTLs. P91A is presented by L^d, P35 by D^d and P198 by K^d.

PCT application PCT/US92/04354, filed on May 22, 1992 assigned to the same assignee as the subject application, teaches a family of human tumor rejection antigen precursor coding genes, referred to as the MAGE family, and their expression in various tumor types. Lung adenocarcinoma is not among these. Several of these genes are also discussed in van der Bruggen et al., Science 254: 1643 (1991). It is now clear that the various genes of the MAGE family are expressed in tumor cells, and can serve as markers for the diagnosis of such tumors, as well as for other purposes discussed therein. See also Traversari et al., Immunogenetics 35: 145 (1992); van der Bruggen et al., Science 254: 1643 (1991). The mechanism by which a protein is processed and presented on a cell surface has now been fairly well documented. A cursory review of the development of the field may be found in Barinaga, "Getting Some 'Backbone': How MHC Binds Peptides", Science 257: 880 (1992); also, see Fremont et al., Science 257: 919 (1992); Matsumura et al., Science 257: 927 (1992); Latron et al., Science 257: 964 (1992). These papers generally point to a requirement that the peptide which binds to an MHC/HLA molecule be nine amino acids long (a "nonapeptide"), and to the importance of the first and ninth residues of the nonapeptide.

Studies on the MAGE family of genes have now revealed that, in some cases a nonapeptide is presented on the surface of tumor cells, and that the presentation of the nonapeptide requires that the presenting molecule be HLA-A1. Complexes of the MAGE-1 tumor rejection antigen (the "TRA" or nonapeptide) leads to lysis of the cell presenting it by cytolytic T cells ("CTLs"). Additional research has correlated other nonapeptides derived from MAGE and genes to HLA-A1 and other MHC class I molecules.

Research presented in, e.g., U.S. patent application Serial No. 07/938,334 filed August 31, 1992, showed that, when comparing homologous regions of various MAGE genes to the region of the MAGE-1 gene coding for the relevant nonapeptide, there is a great deal of homology.

The nucleic acid sequences which code for the nonapeptides were also described therein. These nucleic acid molecules were described as also being useful as diagnostic probes for tumor presence.

The application also showed how it had been found that a cellular model could be used, wherein a non-human cell can be transfected with a nucleic acid sequence coding for a human HLA molecule. The resulting transfectant could then be used to test for nonapeptide specificity of the particular HLA molecule, or as the object of a second transfection with a MAGE gene. The co-transfectant could be used to determine whether the particular MAGE based TRA is presented by the particular HLA molecule.

Many of the references referred to supra present data on the expression pattern of various MAGE genes in different types of cell lines and tumor tissues. What is evident from these data is that there is no "unifying principle" which allows one to predict which MAGE gene will be expressed by a particular tumor type. Thus, while on one level one can say that MAGE genes are "markers" for tumors, on the level of specific tumor types, the correlation of marker and tumor type is not predictable, and must be determined empirically.

It has now been found that one can carry out cancer determination assays by assaying for expression of one or more members of the MAGE family of tumor rejection antigen precursors. How this is accomplished is shown in the examples which follow.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B depict detection of transfectants expressing antigen P815A.

5 Figure 2 shows the sensitivity of clones P1.HTR, PO.HTR, genomic transfectant P1A.T2 and cosmid transfectant P1A.TC3.1 to lysis by various CTLs, as determined by chromium release assays.

Figure 3 is a restriction map of cosmid C1A.3.1.

10 Figure 4 shows Northern Blot analysis of expression of gene P1A.

Figure 5 sets forth the structure of gene P1A with its restriction sites.

15 Figure 6 shows the results obtained when cells were transfected with the gene for P1A, either isolated from P815 or normal cells and then tested with CTL lysis.

Figure 7 shows lytic studies using mast cell line L138. 8A.

Figure 8 is a map of subfragments of the 2.4 kb antigen E fragment which also express the antigen.

20 Figure 9 shows homology of sections of exon 3 from genes mage 1, 2 and 3.

Figure 10 shows the result of Northern blots for MAGE genes on various tissues.

Figure 11 presents the data of Figure 13 in table form.

25 Figure 12 shows Southern Blot experiments using the various human melanoma cell lines employed in this application.

Figure 13 is a generalized schematic of the expression of MAGE 1, 2 and 3 genes by tumor and normal tissues.

Figure 14 shows results from a chromium release assay using CTL clone 20/38 on various cell lines.

5 Figure 15 presents the result of assays undertaken to determine antigenic specificity of CTL clone 20/38.

Figure 16 shows the results obtained when a TNF release assay was carried out on various transfected cells.

10 Figure 17 shows results secured from qualitative PCR assays for MAGE-1 in lung adenocarcinomas.

Figure 18 presents data pertaining to quantitative measurement of MAGE-1 expression in lung adenocarcinomas.

15 Figure 19 shows reverse transcription/PCR amplification production of mRNA extracted from the bladder tumor of a patient referred to as "HM15". This is shown in all lanes marked "R". In lanes marked "D", amplification products of the genomic DNA from the patient are shown.

20 Figure 20 displays the fraction of tumors expressing genes MAGE-1, 2, 3 and 4 among the superficial and invasive transitional cell carcinomas of the bladder.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

25 Many different "MAGE" genes have been identified, as will be seen from the sequences which follow the application. The protocols described in the following examples were used to isolate these genes and cDNA sequences.

"MAGE" as used herein refers to a nucleic acid sequence isolated from human cells. The acronym "smage" is used to describe sequences of murine origin.

When "TRAP" or "TRAs" are discussed herein as being specific to a tumor type, this means that the molecule under consideration is associated with that type of tumor, although not necessarily to the exclusion of other tumor types.

Example 1

In order to identify and isolate the gene coding for antigen P815A, gene transfection was used. This approach requires both a source of the gene, and a recipient cell line. Highly transfectable cell line P1.HTR was the starting material for the recipient, but it could not be used without further treatment, as it presents "antigen A", one of four recognized P815 tumor antigens. See Van Pel et al., Molecular Genetics 11: 467-475 (1985). Thus, screening experiments were carried out to isolate cell lines which did not express the antigen and which nonetheless possessed P1.HTR's desirable qualities.

To do this, P1.HTR was screened with CTLs which were specific for each of tumor antigens A, B, C and D. Such CTLs are described by Uyttenhove et al., J. Exp. Med. 157: 1040-1052 (1983).

To carry out the selection, 10^6 cells of P1.HTR were mixed with $2-4 \times 10^6$ cells of the CTL clone in a round bottom tube in 2 ml of medium, and centrifuged for three minutes at 150xg. After four hours at 37°C, the cells were washed and resuspended in 10 ml of medium, following Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982). Additional information on the CTL assay and screening protocol, in general may be found in Boon et al., J. Exp. Med. 152: 1184-1193 (1980), and Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982), the disclosures of which are incorporated by reference.

When these selections were carried out, a cell line variant was found which expressed neither antigen A or B. Additional selections with CTLs specific for antigen C then yielded a variant which also lacked antigen C. Please see

figure 2 for a summary of the results of these screenings. The variant PO.HTR is negative for antigens A, B and C, and was therefore chosen for the transfection experiments.

5 The cell line PO.HTR has been deposited in accordance with the Budapest Treaty at the Institute Pasteur Collection Nationale De Cultures De Microorganismes, 28, Rue de Docteur Roux, 75724 Paris France, and has accession number I-1117.

10 This methodology is adaptable to secure other cell lines which are variants of a cell type which normally presents at least one of the four recognized P815 tumor antigens, i.e., antigens A, B, C and D, where the variants present none of antigens A, B and C. P1.HTR is a mastocytoma cell line, so it will be seen that the protocol
15 enables the isolation of biologically pure mastocytoma cell lines which express none of P815 antigens A, B and C, but which are highly transfectable. Other tumor types may also be screened in this fashion to secure desired, biologically pure cell lines. The resulting cell lines should be at
20 least as transfectable with foreign DNA as is P1.HTR, and should be selected so as to not express a specific antigen.

Example 2

Previous work reported by DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988) the disclosure of which
25 is incorporated by reference herein had shown the efficacy of using cosmid library transfection to recover genes coding for tum antigens.

Selective plasmid and genomic DNAs of P1.HTR were prepared, following Wölfel et al., Immunogenetics 26: 178-
30 187 (1987). The transfection procedure followed Corsaro et al., Somatic Cell Molec. Genet 7: 603-616 (1981), with some modifications. Briefly, 60 µg of cellular DNA and 3 µg of DNA of plasmid pHMR272, described by Bernard et al., Exp. Cell. Biol. 158: 237-243 (1985) were mixed. This plasmid
35 confers hygromycin resistance upon recipient cells,

and therefore provides a convenient way to screen for transfectants. The mixed DNA was combined with 940 ul of 1 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 310 ul 1M CaCl_2 . The solution was added slowly, and under constant agitation to 1.25 ml of 50 mM Hepes, 280 mM NaCl, 1.5 mM Na_2HPO_4 , adjusted to pH 7.1 with NaOH. Calcium phosphate - DNA precipitates were allowed to form for 30-45 minutes at room temperature. Following this, fifteen groups of PO.HTR cells (5×10^6 per group) were centrifuged for 10 minutes at 400xg. Supernatants were removed, and pellets were resuspended directly into the medium containing the DNA precipitates. This mixture was incubated for 20 minutes at 37°C, after which it was added to an 80 cm² tissue culture flask containing 22.5 ml DMEM, supplemented with 10% fetal calf serum. After 24 hours, medium was replaced. Forty-eight hours after transfection, cells were collected and counted. Transfected cells were selected in mass culture using culture medium supplemented with hygromycin B (350 ug/ml). This treatment selected cells for hygromycin resistance.

For each group, two flasks were prepared, each containing 8×10^6 cells in 40 ml of medium. In order to estimate the number of transfectants, 1×10^6 cells from each group were plated in 5 ml DMEM with 10% fetal calf serum (FCS), 0.4% bactoagar, and 300 ug/ml hygromycin B. The colonies were then counted 12 days later. Two independent determinations were carried out and the average taken. This was multiplied by 5 to estimate the number of transfectants in the corresponding group. Correction had to be made for the cloning efficiency of P815 cells, known to be about 0.3.

Example 3

Eight days after transfection as described in example 2, supra, antibiotic resistant transfectants were separated from dead cells, using density centrifugation with Ficoll-

Paque. These cells were maintained in non-selective medium for 1 or 2 days. The cells were plated in 96 well microplates (round bottom), at 30 cells/microwell in 200 ul of culture medium. Anywhere from 100-400 microwells were prepared, depending on the number of transfectants prepared. Agar colony tests gave estimates of 500-3000. After 5 days, the wells contained about 6×10^4 cells and replicate plates were prepared by transferring 1/10 of the wells to microplates which were then incubated at 30°C. One day later, master plates were centrifuged, medium removed, and 750 CTLs against P815 antigen A (CTL:P1:5) were added to each well together with 10^6 irradiated syngeneic feeder spleen cells in CTL culture medium containing 40 U/ml recombinant human IL-2, and HAT medium to kill stimulator cells. Six days later, plates were examined visually to identify wells where CTLs had proliferated. Where plates showed proliferating microcultures, aliquots of 100 ul of the wells were transferred to another plate containing ^{51}Cr labeled P1.HTR target cells (2×10^3 - 4×10^3 per well), and chromium release was measured after 4 hours. Replicate microcultures corresponding to those showing high CTL activity were expanded and cloned by limited dilution in DMEM with 10% FCS. Five days later, about 200 clones were collected and screened with the CTL.P1:5 cell line, described supra, in a visual lysis assay. See figure 1A for these results.

In these experiments, three of the fifteen groups of transfectants yielded a few positive microcultures. These microcultures were tested for lytic activity against P1.HTR, as described supra. Most of the microcultures where proliferation was observed showed lytic activity. This activity was well above background, as shown in figure 1B. This figure summarizes data wherein two groups of cells (groups "5" and "14"), 400 and 300 microwells were seeded with 30 hygromycin resistant transfected cells. Amplification and duplication of the microcultures was followed by addition of anti-A CTL P1:5. Six days later,

lytic activity against P1.HTR was tested. In the figure, each point represents lytic activity of a single microculture.

5 Duplicate microcultures corresponding to several positive wells were subcloned, and more than 1% of the subclones were found to be lysed by anti-A CTL. Thus, three independent transfectants expressing P815A were obtained from 33,000 hygromycin resistant transfectants. One of these lines, referred to hereafter as P1A.T2 was
10 tested further.

The relevant antigen profile of P1A.T2 is shown in figure 2, this being obtained via anti-CTL assays of the type described supra.

Example 4

15 The CTL assays carried out for P1A.T2 demonstrated that it presented antigen A ("P815A"), and therefore had received the gene from P1.HTR. To that end, this cell line was used as a source for the gene for the antigen precursor in the following experiments.

20 Prior work had shown that genes coding for tum antigens could be recovered directly from transfectants obtained with a cosmid library. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). This procedure was followed for recovery of the P815 gene.

25 Total genomic DNA of P1A.T2 was partially digested with restriction endonuclease Sau 3A1, and fractionated by NaCl density gradient ultracentrifugation to enrich for 35-50 kb DNA fragments, following Grosveld et al., Gene 10: 6715-6732 (1982). These fragments were ligated to cosmid
30 arms of C2RB, described by Bates et al., Gene 26: 137-146 (1983), the disclosure of which is incorporated by reference. These cosmid arms had been obtained by cleavage with SmaI and treatment with calf intestinal phosphatase, followed by digestion with BamHI. Ligated DNA was packaged
35 into lambda phage components, and titrated on E. coli ED 8767, following Grosveld et al., supra. Approximately 9×10^5

ampicillin resistant colonies were obtained per microgram of DNA insert.

The cosmid groups were amplified by mixing 30,000 independent cosmids with 2 ml of ED 8767 in 10 mM MgCl₂,
5 incubated 20 minutes at 37°C, diluted with 20 ml of Luria Bertani ("LB") medium, followed by incubation for one hour. This suspension was titrated and used to inoculate 1 liter of LB medium in the presence of ampicillin (50 ug/ml). At a bacterial concentration of 2×10^8 cells/ml ($OD_{600}=0.8$), a 10
10 ml aliquot was frozen, and 200 ug/ml chloramphenicol was added to the culture for overnight incubation. Total cosmid DNA was isolated by alkaline lysis procedure, and purified on CsCl gradient.

In these experiments, a library of 650,000 cosmids was
15 prepared. The amplification protocol involved the use of 21 groups of approximately 30,000 cosmids.

Example 5

Using the twenty-one groups of cosmids alluded to supra, (60 ug) and 4 ug of pHMR272, described supra, groups
20 of 5×10^6 PO.HTR cells were used as transfectant hosts. Transfection was carried out in the same manner as described in the preceding experiments. An average of 3000 transfectants per group were tested for antigen presentation, again using CTL assays as described. One
25 group of cosmids repeatedly yielded positive transfectants, at a frequency of about 1/5,000 drug resistant transfectants. The transfectants, as with P1A.T2, also showed expression of both antigen A and B. The pattern of expression of transfectant P1A.TC3.1 is shown in figure 2,

Example 6

As indicated in Example 5, supra, three independent cosmid transfected cells presenting P815A antigen were isolated. The DNA of these transfectants was isolated and packaged directly with lambda phage extracts, following
35 DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278

(1988). The resulting product was titrated on E. coli ED 8767 with ampicillin selection, as in Example 5. Similarly, amplification of the cosmids and transfection followed Example 5, again using PO.HTR.

5 High frequencies of transfection were observed, as described in Table 1, which follows:

Table 1. Transfer of the expression of antigen P815A by cosmids obtained by direct packaging

10	Transfectant obtained with the cosmid library	No. of cosmids obtained by direct packaging of 0.5 µg of DNA	No. of transfectants expressing P815A / no. of HmB' transfectants
15	TC3.1	32	87/192
	TC3.2	32000	49/384
	TC3.3	44	25/72

20 The cosmids were analyzed with restriction enzymes and it was found that directly packaged transfectant P1A.TC3.1 contained 32 cosmids, 7 of which were different. Each of these 7 cosmids was transfected into PO.HTR, in the manner described supra, and again, following the protocols described above, transfectants were studied for
25 presentation of P815A. Four of the cosmid transfectants showed P815A presentation and, as with all experiments described herein, P815B was co-expressed.

Of the four cosmids showing presentation of the two antigens, cosmid C1A.3.1 was only 16.7 kilobases long, and
30 was selected for further analysis as described infra.

The cosmid C1A.3.1 was subjected to restriction endonuclease analysis, yielding the map shown in Figure 3.

All EcoRI fragments were transfected, again using the above described protocols, and only the 7.4 kilobase
35 fragment produced a transfectant that anti-A CTLs could lyse. Similar experiments were carried out on the PstI

fragments, and only a 4.1 kb fragment fully contained within the 7.4 kb EcoRI fragment produced lysable transfectants.

5 This fragment (i.e., the 4.1 kb PstI fragment), was digested with SmaI, giving a 2.3 kb fragment which also yielded host cells presenting antigens A and B after transfection. Finally, a fragment 900 bases long, secured with SmaI/XbaI, also transferred expression of the precursors of these two antigens, i.e., the transfected
10 host cell presented both antigen A and antigen B.

Example 7

The 900 base fragment described above was used as a probe to detect the expression of the P815A gene in parent cell line P1.HTR. To accomplish this, total cellular RNA
15 was first isolated using the guanidine-isothiocyanate procedure of Davis et al., Basic Methods In Molecular Biology (Elsevier Science Publishing Co, New York) (1986). The same reference was the source of the method used to isolate and purify polyA⁺ mRNA using oligodT cellulose
20 column chromatography.

Samples were then subjected to Northern Blot analysis. RNA samples were fractionated on 1% agarose gels containing 0.66 M formaldehyde. The gels were treated with 10xSSC (SSC: 0.15 M NaCl; 0.015 M sodium citrate, pH 7.0) for 30
25 minutes before overnight blotting on nitrocellulose membranes. These were baked for two hours at 80°C, after which the membranes were prehybridized for 15 minutes at 60°C in a solution containing 10% dextran sulfate, 1% SDS and 1M NaCl. Hybridization was then carried out using
30 denatured probe (the 900 base fragment), together with 100 ug/ml salmon sperm DNA.

When this protocol was carried out using P1.HTR poly A⁺ RNA, a band of 1.2 kb and two fainter bands were identified, as shown in Figure 4, lane 1 (6 ug of the RNA).

35 The same probe was used to screen a cDNA library, prepared from poly-A⁺ RNA from the cell line. This yielded

a clone with a 1kb insert, suggesting a missing 5' end. The Northern blots for the cDNA are not shown.

Hybridization experiments in each case were carried out overnight at 60°C. The blots were washed twice at room temperature with 2xSSC and twice at 60°C with 2xSSC supplemented with 1% SDS.

The foregoing experiments delineated the DNA expressing the P815A antigen precursor sufficiently to allow sequencing, using the well known Sanger dideoxy chain termination method. This was carried out on clones generated using a variety of restriction endonucleases and by specific priming with synthetic oligonucleotide primers. The results for exons of the gene are set forth in SEQUENCE ID NO: 4.

15 Example 8

The Northern analysis described supra suggested that the 5' end of the cDNA was missing. To obtain this sequence, cDNA was prepared from P1.HTR RNA using a primer corresponding to positions 320-303. The sequence was then amplified using the polymerase chain reaction using a 3' primer corresponding to positions 286-266 and a 5' primer described by Frohman et al., Proc. Natl. Acad. Sci. USA 85: 8998-9002 (1988). A band of the expected size (270 bases) was found, which hybridized to the 900 bp SmaI/XbaI fragment described supra on a Southern blot. Following cloning into m13tg 130 and tg 131, the small, 270 bp fragment was sequenced. The sequence is shown in SEQUENCE ID NO: 1.

Example 9

Following the procurement of the sequences described in Examples 7 and 8 and depicted in SEQ ID NO: 4, a 5.7 kb region of cosmid C1A.3.1 was sequenced. This fragment was known to contain the 900 base fragment which expressed P815A in transfectants. This experiment permitted

delineation of introns and exons, since the cosmid is genomic in origin.

5 The delineated structure of the gene is shown in figure 5. Together with SEQ ID NO: 4, these data show that the gene for the antigen precursor, referred to as "P1A" hereafter, is approximately 5 kilobases long and contains 3 exons. An ORF for a protein of 224 amino acids starts in exon 1, ending in exon 2. The 900 base pair fragment which transfers expression of precursors for antigens A and B only contains exon 1. The promoter region contains a CAAT box, as indicated in SEQ ID NO: 1, and an enhancer sequence. This latter feature has been observed in promoters of most MHC class I genes, as observed by Geraghty et al., J. Exp. Med 171: 1-18 (1990); Kimura et al., Cell 44: 261-272 (1986).

10 A computer homology search was carried out, using program FASTA with K-triple parameters of 3 and 6, as suggested by Lipman et al., Science 227: 1435-1441 (1985), and using Genbank database release 65 (October 1990). No homology was found except for a stretch of 95 bases corresponding to part of an acid region coded by exon 1 (positions 524-618), which is similar to sequences coding for acidic regions in mouse nucleolar protein NO38/B23, as described by Bourbon et al., Mol. Biol. 200: 627-638 (1988), and Schmidt-Zachmann et al., Chromosoma 96: 417-426 (1988). Fifty six of 95 bases were identical. In order to test whether these homologies were the reason for cross hybridizing, experiments were carried out using a mouse spleen cDNA library screened with the 900 base fragment. cDNA clones corresponding closely to the sizes of the cross hybridizing bands were obtained. These were partially sequenced, and the 2.6 kb cDNA was found to correspond exactly to reported cDNA sequence of mouse nucleolin, while the 1.5 kb cDNA corresponded to mouse nucleolar protein NO38/B23.

35 Analysis of the nucleotide sequence of the gene, referred to as "P1A" hereafter, suggests that its coded

product has a molecular mass of 25 kd. Analysis of the
SEQUENCE ID NO: 4 shows a potential nuclear targeting
signal at residues 5-9 (Dingwall et al., Ann. Rev. Cell
Biol. 2: 367-390 (1986)), as well as a large acidic domain
5 at positions 83-118. As indicated supra, this contains the
region of homology between P1A and the two nucleolar
proteins. A putative phosphorylation site can be found at
position 125 (serine). Also, a second acidic domain is
found close to the C-terminus as an uninterrupted stretch
10 of 14 glutamate residues. A similar C-terminal structure
has been found by Kessel et al. Proc. Natl. Acad. Sci. USA
84: 5306-5310 (1987), in a murine homeodomain protein
having nuclear localization.

In studies comparing the sequence of gene P1A to the
15 sequences for P91A, P35B and P198, no similarities were
found, showing that P1A is indicative of a different class
of genes and antigens.

Example 10

With the P1A probe and sequence in hand,
20 investigations were carried out to determine whether the
gene present in normal tissue was identical to that
expressed by the tumor. To do this, phage libraries were
prepared, using lambda zapII 10 and genomic DNA of DBA/2
murine kidney cells. P1A was used as a probe.
25 Hybridization conditions were as described supra, and a
hybridizing clone was found. The clone contained exons one
and two of the P1A gene, and corresponded to positions -0.7
to 3.8 of figure 5. Following localization of this
sequence, PCR amplification was carried out to obtain the
30 sequence corresponding to 3.8 to 4.5 of figure 5.

Sequence analysis was carried out, and no differences
were found between the gene from normal kidneys and the P1A
gene as obtained from the P815 tumor cells.

In further experiments, the gene as found in DBA/2
35 kidney cells was transfected into PO.HTR, as described
supra. These experiments, presented pictorially in figure

6, showed that antigens A and B were expressed as efficiently by the kidney gene isolated from normal P815 cells as with the P1A gene isolated from normal kidney cells.

5 These experiments lead to the conclusion that the gene coding for the tumor rejection antigen precursor is a gene that does not result from a mutation; rather, it would appear that the gene is the same as one present in normal cells, but is not expressed therein. The ramifications of
10 this finding are important, and are discussed infra.

 In studies not elaborated upon herein, it was found that variants of the gene were available. Some cells were "P1A^{B+}", rather than the normal "P1A". The only difference between these is a point mutation in exon 1, with the 18th
15 triplet coding for Ala in the variant instead of Val.

Example 11

 Additional experiments were carried out with other cell types. Following the protocols described for Northern blot hybridizations supra, RNA of normal liver and spleen
20 cells was tested to determine if a transcript of the P1A gene could be found. The Northern blot data are presented in figure 4 and, as can be seen, there is no evidence of expression.

 The murine P815 cell line from which P1A was isolated
25 is a mastocytoma. Therefore, mast cell lines were studied to determine if they expressed the gene. Mast cell line MC/9, described by Nabel et al., Cell 23: 19-28 (1981), and short term cultures of bone marrow derived mast cells were tested in the manner described supra (Northern blotting),
30 but no transcript was found. In contrast when a BALB/C derived IL-3 dependent cell line L138.8A (Hültner et al., J. Immunol. 142: 3440-3446 (1989)) was tested, a strong signal was found. The mast cell work is shown in figure 4.

 It is known that both BALB/C and DBA/2 mice share H-2^d
35 haplotype, and thus it was possible to test sensitivity to lysis using the CTLs described supra. Figure 7 shows these

results, which essentially prove that anti-A and anti-B CTLs lysed the cells strongly, whereas anti-C and anti-D lines did not.

Further tests were carried out on other murine tumor cell lines, i.e., teratocarcinoma cell line PCC4 (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977), and leukemias LEC and WEHI-3B. Expression could not be detected in any of these samples.

Example 12

The actual presentation of the P1A antigen by MHC molecules was of interest. To test this, cosmid C1A.3.1 was transfected into fibroblast cell line DAP, which shows phenotype H-2^k. The cell lines were transfected with genes expressing one of the K^d, D^d, and L^d antigen. Following transfection with both the cosmid and the MHC gene, lysis with CTLs was studied, again as described supra. These studies, summarized in Table 2, show that L^d is required for presentation of the P1A antigens A and B.

Table 2. H-2-restriction of antigens P815A and P815B

	Recipient cell ^a	No. of clones lysed by the CTL/no. of HmB ^a clones ^a	
		CTL anti-A	CTL anti-B
25	DAP (H-2 ^k)	0/208	0/194
	DAP + K ^d	0/165	0/162
	DAP + D ^d	0/157	0/129
	DAP + L ^d	25/33	15/20

^aCosmid C1A.3.1 containing the entire P1A gene was transfected in DAP cells previously transfected with H-2^d class I genes as indicated.

^aIndependent drug-resistant colonies were tested by lysis by anti-A or anti-B CTL in a visual assay.

The observation that one may associate presentation of a tumor rejection antigen with a particular MHC molecule was confirmed in experiments with human cells and HLA molecules, as elaborated upon infra.

5 **Example 13**

 Using the sequence of the P1A gene as well as the amino acid sequence derivable therefrom, antigenic peptides which were A⁺ B⁺ (i.e., characteristic of cells which express both the A and B antigens), and those which are A⁺B⁺ were identified. The peptide is presented in SEQ ID NO: 26. This peptide when administered to samples of PO.HTR cells in the presence of CTL cell lines specific to cells presenting it, led to lysis of the PO.HTR cells, lending support to the view that peptides based on the product expressed by the gene can be used as vaccines.

10
15

Example 14

 The human melanoma cell line referred to hereafter as MZ2-MEL is not a clonal cell line. It expresses four stable antigens recognized by autologous CTLs, known as antigens "D, E, F, and A". In addition, two other antigens "B" and "C" are expressed by some sublines of the tumor. CTL clones specific for these six antigens are described by Van den Eynde et al., Int. J. Canc. 44: 634-640 (1989). Among the recognized subclones of MZ2-MEL are MEL.43, MEL3.0 and MEL3.1. (Van den Eynde et al., supra). Cell line MEL3.1 expresses antigen E, as determined by CTL studies as described for P815 variants, supra, so it was chosen as a source for the nucleic acid sequence expressing the antigen precursor.

20
25

30 In isolating the pertinent nucleic acid molecule for a tumor rejection antigen precursor, the techniques developed supra, showed that a recipient cell is needed which fulfills two criteria: (i) the recipient cell must not express the TRAP of interest under normal conditions,

and (ii) it must express the relevant class I HLA molecule. Also, the recipient cell must have a high transfection frequency, i.e., it must be a "good" recipient.

5 In order to secure such a cell line, the clonal
subline MEL3.1 was subjected to repeated selection with
anti-E CTL 82/30 as described by Van den Eynde, supra. The
repeated cycles of selection led to isolation of subclone
MZ2-MEL-2.2 isc E. This subclone is also HPRT, (i.e.,
sensitive to HAT medium: 10^{-4} M hypoxanthine, 3.8×10^{-7}
10 aminopterin, 1.6×10^{-5} M 2-deoxythymidine). The subclone
is referred to as "MEL-2.2" for simplicity hereafter.

Example 15

The genomic DNA of MEL3.0 was prepared following
Wölfel et al., Immunogenetics 26: 178-187 (1987), the
15 disclosure of which is incorporated by reference. The
plasmid pSVtkneo β , as described by Nicolas et al., Cold
Spring Harb., Conf. Cell Prolif. 10: 469-485 (1983) confers
geneticin resistance, so it can be used as a marker for
cotransfection, as it was in this experiment.

20 Following a procedure similar but not identical to
that of Corsaro et al., Somatic Cell Molec. Genet 7: 603-
616 (1981), total genomic DNA and the plasmid were
cotransfected. The genomic DNA (60 μ g) and plasmid DNA (6
 μ g) were mixed in 940 μ l of 1 mM Tris·HCl (pH 7.5), 0.1 mM
25 EDTA, after which 310 μ l of 1M CaCl_2 was added. This
solution was slowly added, under constant agitation, to
1.25 ml of 2xHBS (50 mM HEPES, 280 mM NaCl 1.5 mM Na_2HPO_4 ,
adjusted to pH 7.1 with NaOH). The calcium phosphate DNA
precipitates were allowed to form for 30-45 minutes at room
30 temperature, after which they were applied to 80 cm^2 tissue
culture flasks which had been seeded 24 hours previously
with 3×10^6 MEL2.2 cells, in 22.5 ml of melanoma culture
medium (Dulbecco's Modified Eagle's Medium) supplemented
with 10% fetal calf serum. After 24 hours, the medium was
35 replaced. Forty eight hours after transfection, the cells

were harvested and seeded at 4×10^6 cells per 80 cm² flask in melanoma culture medium supplemented with 2 mg/ml of geneticin. The geneticin serves as a selection marker.

Example 16

5 Thirteen days after transfection, geneticin-resistant colonies were counted, harvested, and cultured in nonselective medium for 2 or 3 days. Transfected cells were then plated in 96-well microplates at 200 cells/well in 200 μ l of culture medium with 20% fetal calf serum (FCS) in order to obtain approximately 30 growing colonies per well. The number of microcultures was aimed at achieving redundancy, i.e., such that every independent transfectant should be represented at least four times.

10 After 10 days, wells contained approximately 6×10^4 cells. These cells were detached, and 1/3 of each microculture was transferred to a duplicate plate. After 6 hours, i.e., after readherence, medium was removed and 1500 anti-E CTL (CTL 82/30), were added to each well in 100 μ l of CTL culture medium with 35 U/ml of IL-2. One day later, the supernatant (50 μ l) was harvested and examined for TNF concentration, for reasons set forth in the following example.

Example 17

25 The size of the mammalian genome is 6×10^6 kb. As the average amount of DNA integrated in each drug-resistant transfectant was expected to be about 200 kb, a minimum of 30,000 transfectants would need to be examined to ascertain whether antigen E had been transfected. Prior work with murine cells had shown that when a CTL stimulation assay was used, groups containing only 3% of cells expressing the antigen of interest could be identified. This should reduce the number of assays by a factor of 30. While an anti-E CTL assay, as described supra, in mixed E⁺/E⁻ cells was helpful, it was not sufficient in that consistent results could not be obtained.

As a result, an alternative test was devised. Stimulation of CTLs was studied by release of tumor necrosis factor ("TNF") using well known methodologies which need not be repeated here. As described in Example 5, 1500 CTL 82/30 cells had been added per well of transfectants. These CTLs were collected 6 days after stimulation. As indicated supra, after 1/3 of the cells in each well had been removed and the remaining 2/3 (4×10^4) had readhered, the CTLs and IL-2 were added thereto. The 50 μ l of supernatant was removed 24 hours later and transferred to a microplate containing 3×10^4 W13 (WEHI-164 clone 13; Espevik et al., J. Immunol. Meth. 95: 99-105 (1986)) cells in 50 μ l of W13 culture medium (RPMI-1640, supplemented with L-arginine (116 mg/l), L-asparagine (36 mg/l), L-glutamine (216 mg/l), and 10% FCS supplemented with 2 μ g of actinomycin D at 37°C in an 8% CO₂ atmosphere. The cell line W13 is a mouse fibrosarcoma cell line sensitive to TNF. Dilutions of recombinant TNF- β in RPMI 1640 were added to target cell controls.

The W13 cultures were evaluated after 20 hours of incubation, and dead cell percentage was measured using an adaptation of the colorimetric assay of Hansen et al., J. Immunol. Meth. 119: 203-210 (1989). This involved adding 50 ml of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide at 2.5 mg/ml in PBS, followed by two hours of incubation at 37°C. Dark blue formazan crystals were dissolved by adding 100 μ l of lysis solution (1 volume N,N dimethyl formamide mixed at 37°C with two volumes of water containing 30% (w/v) sodium dodecyl sulphate, at pH 4.7 from 1.6% acetic acid and 2.5% 1N HCl). Plates were incubated at 37°C overnight, and ODs were taken at 570 nm using 650 nm as control. Dead cell percentage was determined via the formula:

$$100 \times \left(1 - \frac{100 - (\text{OD}_{570} \text{ sample well})}{\text{OD}_{570} \text{ well} + \text{medium}} \right)$$

following Espevik et al., J. Immunol. Meth. 95: 99-105 (1986). The results showed that even when the ratio of E⁺/E⁻ cells was as low as 1/45, significant production of TNF was observed, thus showing active CTLs. This led to the decision to test the drug resistant transfectants in groups of 30.

Example 18

Cells were tested for TNF production as discussed in Example 17, supra. A total of 100 groups of E⁻ cells (4x10⁶ cells/group) were tested following transfection, and 7x10⁴ independent geneticin resistant transfectants were obtained, for an average of 700 per group. Only one group of transfected cells led to a microculture which caused anti-E antigen CTL clone 82/30 to produce TNF. Of 300 clones tested, 8 were positive. These clones were then tested for lysis by anti-E CTL, using the standard ⁵¹Cr release assay, and were found to be lysed as efficiently as the original E⁺ cell line. The transfectant E.T1, discussed herein, had the same lysis pattern as did MEL2.2 for CTLs against antigens B,C,D and F.

The fact that only one transfectant presented the antigen out of 70,000 geneticin resistance transfectants may at first seem very low, but it is not. The work described supra for P815 showed an average frequency of 1/13,000. Human DNA recipient MEL2.2 appears to integrate 5 times less DNA than P1.HTR.

Example 19

Once transfectant E.T1 was found, analysis had to address several questions including whether an E⁺ contaminant of the cell population was the cause. The analysis of antigen presentation, described supra, shows that E.T1 is B⁺ and C⁺, just like the recipient cell MEL2.2. It was also found to be HPRT⁻, using standard selection

procedures. All E⁺ cells used in the work described herein, however, were HPRT⁺.

It was also possible that an E⁺ revertant of MEL2.2 was the source for E.T1. To test this, the observation by Perucho et al., Cell 22: 309-317 (1980), that cotransfected sequences usually integrate together at a single location of recipient genome was employed. If antigen E in a transfectant results from cotransfection with pSVtkneo β , then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneo β sequences. Wölfel et al., supra, has shown this to be true. If a normally E⁻ cell is transfected with pSVtkneo β , then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneo β sequences. If a normally E⁺ cell transfected with pSVtkneo β is E.T1, however, "co-deletion" should not take place. To test this, the transfectant E.T1 was subjected to immunoselection with 82/30, as described supra. Two antigen loss variants were obtained, which resisted lysis by this CTL. Neither of these had lost geneticin resistance; however, Southern blot analysis showed loss of several neo^r sequences in the variants, showing close linkage between the E gene and neo^r gene in E.T1, leading to the conclusion that E.T1 was a transfectant.

25 Example 20

The E⁺ subclone MZ2-MEL 43 was used as a source of DNA for preparation of a cosmid library. This library of nearly 700,000 cosmids was transfected into MZ2-MEL 2.2 cells, following the cosmid transfection protocols described supra.

By packaging the DNA of cosmid transfectants directly into lambda phage components, it is sometimes possible to retrieve cosmids that contain the sequences of interest. This procedure was unsuccessful here, so rescue of the transfected sequence was accomplished by ligating DNA of

the transfectant to appropriate restriction fragments of cosmid vector pTL6. This was tried with two transfectants and was successful with one of them. One cosmid, referred to as B3, was recovered from this experiment, and subjected to restriction endonuclease digestion via XmaI, or by BamHI digestion of a large, 12 kb XmaI transfected fragment. The fragments were cloned into vector pTZ 18R, and then transfected into MEL2.2. Again, TNF production was the measure by which successful transfection was determined. The experiments led to the determination of a gene sequence capable of transfecting antigen E on the 12 kb XmaI fragment, and then on the 2.4 kb fragment of BamHI digestion of the 12 kb segment.

The 2.4 kb fragment hybridizes with a 2.4 kb fragment from MZ2-MEL and with a T cell clone of patient MZ-2, as determined by Southern Blots (BamHI digested DNA). The band is absent from E antigen loss variants of MZ2-MEL, as seen in SEQ ID NO: 7.

The sequence for the E antigen precursor gene has been determined, and is presented herein:

Example 21

After the 2.4 kb genomic segment had been identified, studies were carried out to determine if an "E+" subline expressed any homologous DNA. Cell line MZ2-MEL 3.0 was used as a source, and a cDNA library was prepared from its mRNA, using art known techniques. The 2.4 kb segment was used as a probe, and an mRNA of about 1.8 kb was identified as homologous, using Northern blot analysis. When cDNA was screened, clones were obtained showing almost complete identity to parts of the 2.4 kb fragment. Two exons were thus identified. An additional exon was located upstream of these, via sequencing segments of cosmid B3 located in front of the 2.4 kb BamHI fragment. The gene extends over about 4.5 kb, as shown in Figure 8. The starting point of the transcribed region was confirmed using PCR for the 5' end of the cDNA. The three exons comprise 65, 73, and 1551

base pairs. An ATG is located at position 66 of exon 3, followed by a 927 base pair reading frame.

Example 22

5 To determine if smaller segments of the 2.4 kb fragment could transfer the expression of antigen E, smaller pieces corresponding to the larger gene were prepared, using art recognized techniques, and transferred into E cells. Figure 8 shows the boundaries of the three segments.

10 Transfer of antigen expression in this manner indicates that the gene codes for the antigen precursor, rather than coding for a protein which activates the antigen.

Example 23

15 The probing of cDNA described supra revealed, surprisingly, two different but closely related cDNAs. These cDNAs, when tested, did not transfer expression of antigen E, but they do show substantial homology to the first cDNA segment. The three segments, appear to indicate
20 a newly recognized family of genes, referred to as "MAGE" for "melanoma antigen". In Figure 9, MAGE-1" directs expression of the antigen from MZ2 cells. Portions of the third exon of each gene are presented in Figure 9. The second and third sequences are more closely related to each
25 other than the first (18.1 and 18.9% difference compared to the first; 12% with each other). Out of 9 cDNA clones obtained, three of each type were obtained, suggesting equal expression. "MAGE" as used hereafter refers to a family of molecules, and the nucleic acids coding for them.
30 These nucleic acids share a certain degree of homology and are expressed in tumor cells including several types of human tumor cells as well as in human tumors. The family is referred to as "MAGE" because the first members were identified in human melanoma cells. As the experiments
35 which follow indicate, however, the members of the MAGE

family are not at all restricted to melanoma tumors; rather, MAGE refers to a family of tumor rejection antigen precursors and the nucleic acid sequences coding therefore. The antigens resulting therefrom are referred to herein as

5 "MAGE TRAs" or "melanoma antigen tumor rejection antigens"

Example 24

Experiments with mouse tumors have demonstrated that new antigens recognized by T cells can result from point mutations that modify active genes in a region that codes

10 for the new antigenic peptide. New antigens can also arise from the activation of genes that are not expressed in most normal cells. To clarify this issue for antigen MZ2-E, the MAGE-1 gene present in the melanoma cells was compared to that present in normal cells of patient MZ2. Amplification

15 by polymerase chain reaction (PCR) of DNA of phytohemagglutinin-activated blood lymphocytes using primers surrounding a 1300 bp stretch covering the first half of the 2.4 kb fragment was carried out. As expected, a PCR product was obtained whereas none was obtained with

20 the DNA of the E⁻ variant. The sequence of this PCR product proved identical to the corresponding sequence of the gene carried by the E⁺ melanoma cells. Moreover, it was found that antigen MZ2-E was expressed by cells transfected with the cloned PCR product. This result suggests that the

25 activation of a gene normally silent is responsible for the appearance of tumor rejection antigen MZ2-E.

Example 25

In order to evaluate the expression of gene MAGE-1 by various normal and tumor cells, Northern blots were

30 hybridized with a probe covering most of the third exon. In contrast with the result observed with human tumor cell line MZ2-MEL 3.0, no band was observed with RNA isolated from a CTL clone of patient MZ2 and phytohemagglutinin-activated blood lymphocytes of the same patient. Also

35 negative were several normal tissues of other individuals

(Figure 10 and Figure 11). Fourteen melanoma cell lines of other patients were tested. Eleven were positive with bands of varying intensities. In addition to these cultured cell lines, four samples of melanoma tumor tissue were analyzed. Two samples, including a metastasis of patient MZ2, proved positive, excluding the possibility that expression of the gene represented a tissue culture artefact. A few tumors of other histological types, including lung tumors were tested. Most of these tumors were positive (Figures 10 and 11). These results indicated that the MAGE gene family is expressed by many melanomas and also by other tumors. However, they provided no clear indication as to which of genes MAGE-1, 2 or 3 were expressed by these cells, because the DNA probes corresponding to the three genes cross-hybridized to a considerable extent. To render this analysis more specific, PCR amplification and hybridization with highly specific oligonucleotide probes were used. cDNAs were obtained and amplified by PCR using oligonucleotide primers corresponding to sequences of exon 3 that were identical for the three MAGE genes discussed herein. The PCR products were then tested for their ability to hybridize to three other oligonucleotides that showed complete specificity for one of the three genes (Figure 9). Control experiments carried out by diluting RNA of melanoma MZ2-MEL 3.0 in RNA from negative cells indicated that under the conditions used herein the intensity of the signal decreased proportionally to the dilution and that positive signals could still be detected at a dilution of 1/300. The normal cells (lymphocytes) that were tested by PCR were confirmed to be negative for the expression of the three MAGE genes, suggesting therefore a level of expression of less than 1/300th that of the MZ2 melanoma cell line (Figure 11). For the panel of melanoma cell lines, the results clearly showed that some melanomas expressed MAGE genes mage 1, 2 and 3 whereas other expressed only mage-2 and 3 (Figures 11 and 10). Some of the other tumors also

expressed all three genes whereas others expressed only
MAGE-2 and 3 or only MAGE-3. It is impossible to exclude
formally that some positive PCR results do not reflect the
expression of one of the three characterized MAGE genes but
5 that of yet another closely related gene that would share
the sequence of the priming and hybridizing oligo-
nucleotides. It can be concluded that the MAGE gene family
is expressed by a large array of different tumors and that
these genes are silent in the normal cells tested to this
10 point.

Example 26

The availability of a sequence that transfects at high
efficiency and efficiently expresses a TRAP made it
possible to search for the associated major
15 histocompatibility complex (MHC) class I molecule. The
class I specificities of patient MZ2 are HLA-A1, A29, B37,
B44 and C6. Four other melanomas of patients that had A1
in common with MZ2 were cotransfected with the 2.4 kb
fragment and pSVtkneo β . Three of them yielded neo'
20 transfectants that stimulated TNF release by anti-E CTL
clone 82/30, which is CD8+ (Figure 11). No E- transfectant
was obtained with four other melanomas, some of which
shared A29, B44 or C6 with MZ2. This suggests that the
presenting molecule for antigen MZ2-E is HLA-A1. In
25 confirmation, it was found that, out of 6 melanoma cell
lines derived from tumors of HLA-A1 patients, two
stimulated TNF release by anti-E CTL clone 82/30 of patient
MZ2. One of these tumor cell lines, MI13443-MEL, also
showed high sensitivity to lysis by these anti-E CTL,
30 These two melanomas were those that expressed MAGE-1 gene
(Figure 11). Eight melanomas of patients with HLA
haplotypes that did not include A1 were examined for their
sensitivity to lysis and for their ability to stimulate TNF
release by the CTL. None was found to be positive. The
35 ability of some human anti-tumor CTL to lyse allogeneic
tumors sharing an appropriate HLA specificity with the

original tumor has been reported previously (Darrow, et al., J. Immunol. 142: 3329 (1989)). It is quite possible that antigenic peptides encoded by genes MAGE 2 and 3 can also be presented to autologous CTL by HLA-A1 or other class I molecules, especially in view of the similar results found with murine tumors, as elaborated upon supra.

Example 27

As indicated supra, melanoma MZ2 expressed antigens F, D and A', in addition to antigen E. Following the isolation of the nucleic acid sequence coding for antigen E, similar experiments were carried out to isolate the nucleic acid sequence coding for antigen F.

To do this, cultures of cell line MZ2-MEL2.2, an E cell line described supra, were treated with anti-F CTL clone 76/6, in the same manner described for treatment with anti-E CTL clones. This resulted in the isolation of an F antigen loss variant, which was then subjected to several rounds of selection. The resulting cell line, "MZ2-MEL2.2.5" was completely resistant to lysis by anti-F CTLs, yet proved to be lysed by anti-D CTLs.

Again, following the protocols set forth for isolation of antigen -E precursor DNA, the F⁻ variant was transfected with genomic DNA from F⁺ cell line MZ2-MEL3.0. The experiments yielded 90,000 drug resistant transfectants. These were tested for MZ2-F expression by using pools of 30 cells in the TNF detection assay elaborated upon supra. One pool stimulated TNF release by anti-F CTLs, and was cloned. Five of 145 clones were found to stimulate anti-F CTLs. Lysis assays, also following protocols described supra, confirmed (i) expression of the gene coding for antigen F, and (ii) presentation of antigen F itself.

Example 28

Following identification of F⁺ cell lines, the DNA therefrom was used to transfect cells. To do this, a cosmid library of F⁺ cell line MZ2-MEL.43 was prepared,

again using the protocols described supra. The library was divided into 14 groups of about 50,000 cosmids, and DNA from each group was transfected into MZ2-MEL2.2.5. Transfectants were then tested for their ability to stimulate TNF release from anti-F CTL clone 76/6. Of 14 groups of cosmids, one produced two independent transfectants expressing antigen F; a yield of two positives out of 17,500 genitacin resistant transfectants.

Example 29

The existence of a gene family was suggested by the pattern observed on the Southern blot (Figure 12). To do this, the 2.4 kb BamHI fragment, which transferred the expression of antigen MZ2-E, was labelled with ^{32}p and used as a probe on a Southern Blot of BamHI digested DNA of E + cloned subclone MZ2-MEL2.2. Hybridization conditions included $50\text{ }\mu\text{l}/\text{cm}^2$ of 3.5xSSC, 1xDenhardt's solution; 25 mM sodium phosphate buffer (pH 7.0), 0.5% SDS, 2mM EDTA, where the 2.4 kb probes had been labelled with [$\alpha^{32}\text{p}$]dCTP (2-3000 Ci/mole), at 3×10^6 cpm/ml. Hybridization was carried out for 18 hours at 65°C. After this, the membranes were washed at 65°C four times for one hour each in 2xSSC, 0.1% SDS, and finally for 30 minutes in 0.1xSSC, 0.1% SDS. To identify hybridization, membranes were autoradiographed using Kodak X-AR film and Kodak X-Omatic fine intensifying screens.

In the following examples, whenever "hybridization" is referred to, the stringency conditions used were similar to those described supra. "Stringent conditions" as used herein thus refers to the foregoing conditions; subject to routine, art recognized modification.

Example 30

The cDNA coding for MAGE 4 was identified from a sample of the human sarcoma cell line LB23-SAR. This cell line was found to not express MAGE 1, 2 or 3, but the mRNA of the cell line did hybridize to the 2.4 kb sequence for

MAGE 1. To study this further, a cDNA library was prepared from total LB23-SAR mRNA, and was then hybridized to the 2.4 kb fragment. A cDNA sequence was identified as hybridizing to this probe, and is identified hereafter as MAGE 4.

Example 31

Experiments were carried out using PHA-activated lymphocytes from patient "MZ2", the source of the "MZ" cells discussed supra. An oligonucleotide probe which showed homology to MAGE 1 but not MAGE 2 or 3 was hybridized with a cosmid library derived from the PHA activated cells. The size of the hybridizing BamHI cosmid fragment, however, was 4.5 kb, thus indicating that the material was not mage 1; however, on the basis of homology to mage 1-4, the fragment can be referred to as "MAGE 5". The sequence of MAGE 5 is presented in SEQ ID NO: 16.

Example 32

Melanoma cell line LB-33-MEL was tested. Total mRNA from the cell line was used to prepare cDNA, which was then amplified with oligos CHO9: (ACTCAGCTCCTCCCAGATTT) (SEQ ID NO: 53), and CHO10: (GAAGAGGAGGGGCCAAG) (SEQ ID NO: 54). These oligos correspond to regions of exon 3 that are common to previously described mage 1, 2 and 3.

To do this, 1 μ g of RNA was diluted to a total volume of 20 μ l, using 2 μ l of 10x PCR buffer, 2 μ l of each of 10 mM dNTP, 1.2 μ l of 25 mM $MgCl_2$, 1 μ l of an 80 mM solution of CHO9, described supra, 20 units of RNAsin, and 200 units of M-MLV reverse transcriptase. This was followed by incubation for 40 minutes at 42°C. PCR amplification followed, using 8 μ l of 10x PCR buffer, 4.8 μ l of 25 mM $MgCl_2$, 1 μ l of CHO10, 2.5 units of *Thermus aquaticus* ("Taq") polymerase, and water to a total volume of 100 μ l. Amplification was then carried out for 30 cycles (1 minute 94°C; 2 minutes at 52°C, 3 minutes at 72°C). Ten μ l of each reaction were then size fractionated on agarose gel,

followed by nitrocellulose blotting. The product was found to hybridize with oligonucleotide probe CHO18 (TCTTGATCCTGGAGTCC) (SEQ ID NO: 55). This probe identified mage 1 but not mage 2 or 3. However, the product did not hybridize to probe SEQ 4 (TTGCCAAGATCTCAGGAA) (SEQ ID NO: 56). This probe also binds mage 1 but not 2 and 3. This indicated that the PCR product contained a sequence that differed from MAGE 1, 2 and 3. Sequencing of this fragment also indicated differences with respect to MAGE 4 and 5. These results indicate a sequence differing from previously identified MAGE 1, 2, 3, 4 and 5, and is named MAGE 6.

Example 33

In additional experiments using cosmid libraries from PHA-activated lymphocytes of MZ2, the 2.4 kb MAGE 1 fragment was used as a probe and isolated a complementary fragment. This clone, however, did not bind to oligonucleotides specific for MAGE 1, 2, 3 or 4. The sequence obtained shows some homology to exon 3 of mage 1, and differs from MAGES 1-6. It is referred to as mage 7 hereafter. Additional screenings yielded MAGE 8-11. All MAGE sequences identified are presented as SEQ ID's.

Example 34

The usefulness of the TRAPs, as well as TRAs derived therefrom, was exemplified by the following.

Exon 3 of MAGE 1 was shown to transfer expression of antigen E. As a result, it was decided to test whether synthetic peptides derived from this exon 3 could be used to confer sensitivity to anti-E CTL.

To do this, and using standard protocols, cells normally insensitive to anti-E/CTLs were incubated with the synthetic peptides derived from Exon 3.1. Using the CTL lytic assays described supra on P815A, and a peptide concentration of 3 mM, the peptide Glu-Ala-Asp-Pro-Thr-Gly-His-Ser-Tyr (SEQ ID NO: 26) was shown to be best. The

assay showed lysis of 30%, indicating conferring of sensitivity to the anti-E CTL.

Example 35

5 Nucleic acid sequences referred to as "smage" were isolated from murine cells. Using the protocols described supra, a cosmid library was prepared from the DNA of normal DBA/2 kidney cells, using cosmid vector C2RB. As a probe, the 2.4 kb BamHI fragment of MAGE-1 was used. The DNA was
10 blotted to nylon filters, and these were washed in 2xSSC at 65°C to identify the smage material.

Example 36

Further tissue samples were tested for the presence of MAGE genes, using the protocols discussed supra. Some of these results follow.

15 There was no expression of the MAGE genes in brain or kidney tumor tissue. Colon tumor tissue showed expression of MAGE 1, 2, 3 and 4, although not all tumors tested showed expression of all MAGE genes. This is also true for pancreatic tumor (MAGE 1); non-small cell lung (MAGE 1, 2,
20 3 and 4), prostate (MAGE 1), sarcomas (MAGE 1, 2, 3 and 4), breast (MAGE 1, 2 and 3), and larynx (MAGE 1 and 4).

Example 37

A cytolytic CTL clone "20/38" was obtained from peripheral blood lymphocytes of melanoma patient MZ2. This
25 clone is described by Van den Eynde et al., Int. J. Cancer 44: 634-640 (1989), the disclosure of which is incorporated by reference. The CTL clone was isolated following Herin et al., Int. J. Cancer 39: 390-396 (1987), which is incorporated by reference. The assay is described herein,
30 however. Autologous melanoma cells were grown in vitro, and then resuspended at 10^7 cells/ml in DMEM, supplemented with 10% HEPES and 30 mM FCS, and incubated for 45 minutes at 37°C with 200 μ Ci/ml of $\text{Na}^{(51)\text{Cr}}\text{O}_4$. Labelled cells were washed three times with DMEM, supplemented with 10 mM

HEPES. These were then resuspended in DMEM supplemented with 10 mM HEPES and 10% FCS, after which 100 μ l aliquots containing 10^3 cells, were distributed into 96 well microplates. Samples of the CTL clone were added in 100 μ l of the same medium, and assays were carried out in duplicate. Plates were centrifuged for four minutes at 100g, and incubated for four hours at 37°C in a 5.5% CO₂ atmosphere.

Plates were centrifuged again, and 100 μ l aliquots of supernatant were collected and counted. Percentage of ⁵¹Cr release was calculated as follows:

$$\% \text{ } ^{51}\text{Cr release} = \frac{(\text{ER}-\text{SR})}{(\text{MR}-\text{SR})} \times 100$$

where ER is observed, experimental ⁵¹Cr release, SR is spontaneous release measured by incubating 10^3 labeled cells in 200 μ l of medium alone, and MR is maximum release, obtained by adding 100 μ l 0.3% Triton X-100 to target cells.

Those mononuclear blood samples which showed high CTL activity were expanded and cloned via limiting dilution, and were screened again, using the same methodology.

The same method was used to test target K562 cells. When EBV-B cells were used, the only change was the replacement of DMEM medium by Hank's medium, supplemented with 5% FCS.

These experiments led to isolation of CTL clone 20/38.

Figure 14 presents the results of these assays. Specifically, it will be seen that the CTL clone lysed autologous melanoma cell line MZ2-MEL.3.0, but did not lyse EBV-B cell lines, fibroblasts, K562 or non-autologous melanoma cell line SK-MEL-29.

Example 38

Once the CTL clone was recognized as being specific for the autologous cell line, it was tested for antigenic

specificity. To do this, antigen loss variants derived from melanoma cell line MEL-MZ2 were tested in the same type of chromium release assay described above. These target lines were MZ2-MEL 3.0, which is D⁺, E⁺, F⁺, A⁺, MZ2-MEL.61, which is D⁻, MZ2-MEL 2.2, which is E⁻, and MZ2-MEL.4, which is F⁻. In addition to CTL clone 20/38, clones which are known to be anti-A (CTL 28/336), anti-F (CTL 76/6), and anti-E (CTL 22/13) were tested.

These results are set forth in figure 15. It will be seen that CTL clone 20/38 lysed all the cell lines leading to chromium release except D⁻ cell line MZ2-MEL.61, thus indicating that the CTL clone is anti-D. This result was confirmed, in experiments not included herein, by experiments where TNF release by the CTL clone was observed only in the presence of melanoma lines presenting antigen D.

Example 39

Once antigen D was identified as the target molecule, studies were carried out to determine the HLA type which presented it. The experiments described in example 38 showed that antigen D was presented by MZ2-MEL, and this cell line's HLA specificity is known (i.e., A1, A29, B37, B44, Cw6, C.cl.10). It was also known, however, that a variant of MZ2-MEL which had lost HLA molecules A29, B44 and C.cl.10 still expressed antigen D, so these could be eliminated from consideration. Studies were not carried out on lines expressing B37, as none could be found.

In all, 13 allogeneic lines were tested, which expressed either HLA-A1 (10 of 13), or Cw6 (3 of 13). The cell lines were tested for their ability to stimulate release of TNF by CTL clone 20/38, using the method of Traversari et al., Immunogenetics 35: 145-152 (1992), the disclosure of which is incorporated by reference. This assay measures TNF release via testing toxicity of supernatants on WEHI 164-13 cells.

In the assays, cell samples (3000, 10,000 or 30,000 cells) from the allogeneic lines were cultured in the presence of 1500 cells of the CTL clone, and 25 u/ml of IL-2. Twenty-four hours later, the supernatant from the culture was tested against the WEHI cells for toxicity. The results are presented in Table 3, which follows.

Eight cell lines were found to stimulate TNF release from the CTL clone 20/38. All of these lines were HLA-A1. None of the Cw6 presenting lines did so.

The cell lines were also assayed to determine MAGE expression. All eight of the lines which stimulated TNF release expressed MAGE-3, whereas the two HLA-A1 lines which were negative did not.

Table 3

Melanoma	Number of Cells	TNF pg/ml		Expression of Mage-3		Expression of HLA-A-1	
		Exp 1		Exp 2			
		+CTL 20/38	+CTL 20/38				
M22-MEL.61.2	50000	1	4	+++	+		
M22-MEL-ET1	50000	>120	>120	+++	+		
	1666	66	>120				
LY-1-MEL	30000	1	>120	1	>120	+++	+
	10000	1	>120	1	>120		
	3000	<1	114	2	>120		
MI-10221	30000	<1	>120			+++	+
	10000	<1	71				
	3000	<1	74				
LY-2-MEL	30000	1	57			+++	+
	10000	1	86				
	3000	1	91				
LY-4-MEL	30000	1	>120			+++	+
	10000	1	>120				
	3000	1	>120				
SK23-MEL	30000	1	112			+++	+
	10000	1	116				
	3000	1	105				
MI-665/2-MEL	30000	1	3	2	4	-	+
	10000	1	2	2	5		
	3000	1	5,2	1	5		
LB34-MEL	30000	1	>120			+++	+
	10000	1	>120				
	3000	1	>120				
LB45-MEL	30000	1	11	1	30	-	+
	10000	1	6	1	12		
	3000	1	2	<1	7		
NA-6-MEL	30000	1	77	5	98	+++	+
	10000	1	104	5	>120		
	3000	1	110	4	>120		
MI-13443-MEL	30000	1	>120			+++	+
	10000	1	>120				
	3000	1	>120				
LB5-MEL	30000	1	8	4	9	+	-
	10000	<1	5	4	11		
	3000	<1	5	1	5		
SK64-MEL	30000	1	4	2	5	?	-
	10000	1	2	1	5		
	3000	1	1	1	4		
LB33-MEL	30000			1	3,5	+++	-
	10000			1	4		
	3000			1	3		
LB73-MEL	50000		16			-	-

1500 CTL 20/38 and 25 μ /ml IL2 were mixed with the indicated number of cells of the different allogeneic melanomas. 24 hours later, the amount of TNF present in the supernatant was assayed by testing its cytotoxicity for WEHI-164-13 cells.

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Example 40

In view of the results set forth in example 39, experiments were carried out to determine if antigen D was in fact a tumor rejection antigen derived from MAGE-3. To do this, recipient COS-7 cells were transfected with 100ng of the gene for HLA-A1 cloned into pcDNA I/Amp, and 100 ng of one of (a) cDNA for MAGE-1 cloned into pcDNA I/Amp, (b) cDNA for MAGE-2 cloned into pcDSR α , or (c) cDNA for MAGE-3 cloned into pcDSR α . The transfecting sequences were ligated into the plasmids in accordance with manufacturer's instructions. Samples of COS-7 cells were seeded, at 15,000 cells/well into tissue culture flat bottom microwells, in Dulbecco's modified Eagles Medium ("DMEM") supplemented with 10% fetal calf serum. The cells were incubated overnight at 37°C, medium was removed and then replaced by 30 μ l/well of DMEM medium containing 10% Nu serum, 400 μ g/ml DEAE-dextran, 100 μ M chloroquine, and the plasmids described above. Following four hours of incubation at 37°C, the medium was removed, and replaced by 50 μ l of PBS containing 10% DMSO. This medium was removed after two minutes and replaced by 200 μ l of DMEM supplemented with 10% of FCS.

Following this change in medium, COS cells were incubated for 24 hours at 37°C. Medium was then discarded, and 1500 cells of CTL clone 20/38 were added, in 100 μ l of Iscove's medium containing 10% pooled human serum, supplemented with 25 u/ml of IL-2. Supernatant was removed after 24 hours, and TNF content was determined in an assay on WEHI cells, as described by Traversari et al., Immunogenetics 35: 145-152 (1992), the disclosure of which is incorporated by reference. These results are shown in figure 16.

It will be seen that the CTL clone was strongly stimulated by COS-7 cells transfected with HLA-A1 and MAGE-3, but not by the cells transfected with the other Mage genes. This leads to the conclusion that antigen D is a tumor rejection antigen derived from the tumor rejection

antigen precursor coded by gene MAGE-3, and that this TRA is presented by HLA-A1 molecules.

Example 41

5 It is well known that different alleles of genes may produce different proteins. This principle should extend to the MAGE family of genes as well, and is an important consideration in view of diagnostic and therapeutic ramifications. Thus, polymorphism in the MAGE family was studied.

10 To address the issue of polymorphism, blood lymphocytes of ten individuals were collected, and genomic DNA extracted. This DNA was subjected to Southern blotting in accordance with James et al., Canc. Res. 48: 5546-5551 (1988), incorporated by reference. Briefly, the labelled
15 2.4 kb genomic DNA fragment of MAGE-1, containing the last two exons of MAGE-1, described supra, was hybridized with the filter carrying the digested DNA, at 42°C for at least 16 hours, in 50% formamide, 5% dextran sulfate, 6xSSC, 1% SDS and 0.1 mg/ml heterologous DNA. The hybridization
20 filters were washed, consecutively, in 2xSSC, 0.1% SDS (room temperature, 15 minutes), and twice in 0.1xSSC, 0.1% SDS at 67°C for 30 minutes, each wash. Autoradiography was carried out at -70°C for 7-10 days, using standard film.

25 A pattern of 13 hybridizing bands was observed, which was conserved over all individuals. One individual did show an additional band, but also showed the 13 band pattern.

Example 42

30 It was of interest to determine which chromosome or chromosomes bear the MAGE genes. To ascertain this, a panel of hamster/human somatic cell hybrids was used. The hybrids were obtained either from the Human Genetic Mutant Cell Repository ("GM" prefix), or from Johns Hopkins University ("A₃" prefix). Each hybrid was cytogenetically
35 studied to determine human chromosome content.

Total genomic DNA of the hybrids was probed in the same manner described in Example 41, supra (the conditions of stringency used prevented cross hybridization with hamster DNA).

- 5 Table 4, which follows, summarizes the result of the probe work. . Analysis of the data led to the conclusion that the pattern of hybridization was only concordant with location of MAGE-1 on the X chromosome.

TABLE 4 - Segregation of MAGE-1 with human chromosomes in human-hamster hybrid cell DNA

Hybrid	MAGE-1	Human chromosome																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
GM06317	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
GM06318B	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM07300	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
GM07301	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
GM08854	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
GM09142	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	2	-
GM10095	+	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	4	-
GM10115	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10156B	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10253	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10322	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10478	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
GM10479	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
GM10498	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
GM10567	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
GM10611	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10612	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
GM10629	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10791	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10880	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
GM10888	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A1ADA ID12	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	+	-	-	-	-	+	-	+	-	-
A3ADA6F5	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A1ADA13	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	±	-	+	+	-	+	+	+	+
A1ADA14	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	±	-	-	-	+	-	+	+	+	+
A3GI	+	-	-	+	+	+	6	-	±	+	+	+	+	+	+	±	-	-	±	+	+	±	+	+	+
A1HR20	-	-	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-	-	±	+	+
PgMe4	+	-	5	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	±	+	±	±	±	±
Number of concordant hybrids	(+/+) (-/-)	2 17	1 19	3 14	2 14	3 15	3 18	4 16	2 17	1 18	3 17	4 16	3 17	3 14	3 18	3 17	0 17	3 17	1 19	2 18	1 16	3 15	1 14	8 15	4 17
Number of discordant hybrids	(+/+) (-/-)	6 2	6 0	5 5	6 4	7 5	3 2	5 1	3 3	6 2	7 1	5 2	4 2	5 3	5 5	4 0	7 2	5 2	7 0	5 1	6 3	5 5	6 3	0 0	4 2
Percent discordancy		22	23	38	37	41	24	22	23	30	30	26	22	30	37	16	33	26	27	23	35	37	36	0	26

+ = chromosome present; - = chromosome absent;

± = very faint bands, indicating that only a small percentage of the cells contained the chromosome (not included in calculation of percent discordancy)

1,2 - GM09142 contains only part of chromosomes X and 21, der 21 1(X;21)(p21;p12)

3,4 - GM10095 contains only part of chromosomes X and 9, der 9 1(X;9)(q13;q34)

5 - PgMe4 contains a deleted chromosome 2 and is missing 2p23-p24

6 - A3GI contains only the q arm of chromosome 6

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Example 43

In this experiment, a study was carried out to determine if all twelve known MAGE genes were located on the X chromosome. This was accomplished via the use of polymerase chain reaction ("PCR") technology.

RNA purification and cDNA synthesis were first carried out, in accordance with Weynants et al., Int. J. Cancer 56: 826-829 (1994), incorporated by reference herein. Next, 1/20 of the cDNA produced from 2 ug of total RNA was supplemented with 5 ul of PCR buffer (500 mM KCl, 100 mM Tris pH 8.3), 1 ul each of 10 mM dNTPs, 25 pmoles of each primer (see below), 3 ul of 25 mM MgCl₂, and 1.25 units of Taq polymerase, with water added to final volume of 50 ul.

The primers were as follows:

- 15 MAGE-3: 5'-TGGAGGACCAGAGGCCCCC, 5'-GGACGATTATCAGGAGGCCTGC (725 bp) (SEQ ID NOS: 27 AND 28)
MAGE-4: 5'-GAGCAGACAGGCCAACCG, 5'-AAGGACTCTGCGTCAGGC (446 bp) (SEQ ID NOS: 29 AND 30)
MAGE-5: 5'-CTAGAGGAGCACCAAAGGAGAAG, 5'-TGCTCGGAACACAGACTCTGG (413 bp) (SEQ ID NOS: 31 AND 32)
20 MAGE-6: 5'-TGGAGGACCAGAGGCCCCC, 5'-CAGGATGATTATCAGGAAGCCTGT (727 bp) (SEQ ID NOS: 33 AND 34)
MAGE-7: 5'-CAGAGGAGCACCGAAGGAGAA, 5'-CAGGTGAGCGGGGTGTGTC (405 bp) (SEQ ID NOS: 35 AND 36)
25 MAGE-8: 5'-CCCCAGAGAAGCACTGAAGAAG, 5'-GGTGAGCTGGGTCCGGG (399 bp) (SEQ ID NOS: 37 AND 38)
MAGE-9: 5'-CCCCAGAGCAGCACTGACG, 5'-CAGCTGAGCTGGGTCCGACC (391 bp) (SEQ ID NOS: 39 AND 40)
MAGE-10: 5'-CACAGAGCAGCACTGAAGGAG, 5'-CTGGGTAAAGACTCACTGTCTGG (485 bp) (SEQ ID NOS: 41 AND 42)
30 MAGE-11: 5'-GAGAACCCAGAGGATCACTGGA, 5'-GGGAAAAGGACTCAGGGTCTATC (422 bp) (SEQ ID NOS: 43 AND 44)
MAGE-12: 5'-GGTGAAGTGGTCCGCATCG, 5'-GCCCTCCACTGATCTTTAGCAA (392 bp) (SEQ ID NOS: 45 AND 46)
35 Amplification was carried out for 30 cycles (MAGE-3, 4, 6, 12) or 32 cycles (MAGE-5, 7-11), where a cycle was one minute at 94°C followed by two minutes at 65°C for MAGE-5, 7-12, or two

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minutes at 68°C (MAGE-4), or two minutes at 71°C (MAGE-3 and MAGE-6); followed by three minutes at 72°C (MAGE-3, 5-12), or two minutes at 72°C (MAGE-4). The analysis was carried out on hybrid cell line GM 10868, which contains human chromosome 12, and GM 07301, which contains chromosome 12 and the X-chromosome. All assays were negative with the human GM 10868 line, and all were positive with the GM 07301 cell line, which indicated that all 12 genes are found on the X-chromosome.

Example 44

The sizes of mRNAs for the different MAGE genes are similar, and thus Northern blot analysis cannot be used to determine expression of the various MAGE genes in different tissues, both normal and tumor. PCR analysis, along the lines of the study in example 43, supra, however, was believed to be useful.

To this end, a series of various tumors and normal tissues were tested for expression of MAGE genes.

Total RNA of the cells tested was extracted, and was then oligo dT primed, following art known techniques. The resulting material was then subjected to PCR, following the protocols of example 43, supra. For MAGE-1 and MAGE-2, the protocols of Brasseur et al., Int. J. Cancer. 52: 839-841 (1992), and DeSmet et al., Immunogenetics 39: 121-120 (1994), both of which are incorporated by reference, were used.

Table 5, which follows, elaborates these results, with a representative but by no means exhaustive listing of tissues tested. Each of MAGE 1-4, 6 and 12 showed significant expression in a number of tumors of varied tissue types. MAGE-5 and 8-11 were expressed very weakly in all tissues tested, whereas MAGE-7 RNA was not detectable at all. With respect to normal tissues, including tissues taken from a >20 week fetus, all were negative for MAGE RNA but for testis and placenta. Testis expressed all MAGE genes but MAGE-7, while placenta expressed MAGE-3, 4, and 8-11.

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TABLE 5. Expression of Mage-1, 2, 3, 4, 6 and -12 by tumors and normal tissues

	MAGE 1	MAGE 2	MAGE 3	MAGE 4	MAGE 6	MAGE 12
COLON CARCINOMAS						
MZ-CO-2 ¶	++	++	+	-	-	+
SK-CO-11 ¶	-	++	+++	-	+	++
LB150 **	-	-	-	+	-	-
HSR 320 ¶	-	+++	+++	+	++	+++
LEUKEMIAS						
K562 ¶	-	++	+++	-	++	+++
MELANOMAS						
MI10221 ¶	-	+++	+++	+++	+++	+++
MZ2-MEL 3.0 ¶	+++	+++	+++	-	+++	+
LB265 **	-	++	-	-	-	+
LG7 **	-	++	-	-	-	-
LG11 **	++	++	++	-	-	+++
LB271 **	-	++	+++	-	++	+++
LUNG CANCERS						
LB178 (NSCLC) **	++	-	-	+++	-	-
LB175 (NSCLC) **	-	++	+++	+++	-	+++
LB11 (SCLC) ¶	++	+++	+++	-	-	+++
LB12 (SCLC) ¶	-	+++	+++	-	-	+++
SARCOMAS						
LB23 ¶	-	-	-	++	-	-
LB408 **	-	-	-	++	-	-
LS258 **	+	++	+	-	-	++
BREAST CARCINOMAS						
LB280 **	++	-	++	-	-	+
LS284 **	++	++	++	+	-	++
Normal Tissues						
Stomach	-	-	-	-	-	-
Lung	-	-	-	-	-	-
Breast	-	-	-	-	-	-
Colon	-	-	-	-	-	-
Skin	-	-	-	-	-	-
Uterus	-	-	-	-	-	-
Testis	++	++	++	++	++	++
Thymocytes	-	-	-	-	-	-
EBV-lymphocytes	-	-	-	-	-	-
Foetal liver	-	-	-	-	-	-
Foetal brain	-	-	-	-	-	-
Placenta LB694	-	-	+	+++	-	-

RNA from tumor cell lines (¶), tumor samples (**) and normal tissues were tested by RT-PCR for the expression of MAGE genes. PCR primers were chosen as indicated in methods. For MAGE-12, PCR amplification of RNA in the absence of reverse transcription indicated that in our conditions the contamination by genomic DNA was negligible. The level of expression evaluated by band intensity of PCR products fractionated in agarose gels is represented by +++, ++, +. Absence of product is indicated by -.

Example 45

The expression of the MAGE-1, 2 and 3 genes in various tumors and normal tissues was evaluated, using both reverse transcription and polymerase chain reaction ("PCR") amplification. To perform these assays, the total RNA of the cells of interest was extracted via the well known guanidine-isothiocyanate procedure of Davis et al., Basic Methods in Molecular Biology, 1986 (New York, Elsevier, pp. 130), which is incorporated by reference in its entirety. cdNA was then synthesized, by taking 2 ug of the RNA, diluting it with water, and then adding the following materials: 4 ul of 5X reverse transcriptase buffer, 1 ul each of each dNTP (10 mM), 1 ul of a 40 μ M solution of oligo dT(15), 20 units of RNAsin, 2 ul of 0.1 M dithiothreitol, and 200 units of MoMLV reverse transcriptase. All materials were mixed in a 20 ul reaction volume, and incubated at 42°C for 60 minutes and diluted to 100 ul with water.

Presence or absence of each of MAGE-1, -2, and -3 cdNA was detected via PCR amplification, in separate reactions, using oligonucleotide primers located in different exons of the MAGE gene of interest. For MAGE-1, the primers were:

5'-CGGCCGAAGGAACCTGACCCAG-3'
(SEQ ID NO: 47)

5'-GCTGGAACCCTCACTGGGTTGCC-3'
(SEQ ID NO: 48)

These are described by Brasseur et al., Int. J. Cancer 52: 839-841 (1992).

For MAGE-2, the primers were:

5'-AAGTAGGACCCGAGGCACTG-3'
(SEQ ID NO: 49)

5'-GAAGAGGAAGAAGCGGTCTG-3'
(SEQ ID NO: 50)

(DeSmet et al., Immunogenetics 39: 121-129 (1994)).

For MAGE-3, the primers were:

5'-TGGAGGACCAGAGGCCCCC-3'
(SEQ ID NO: 27)

5
5'-GGACGATTATCAGGAGGCCTGC-3'
(SEQ ID NO: 28)

(Serial No. 08/204,727 filed March 1, 1994 to Gaugler et al. incorporated by reference).

10 For each PCR reaction, 5 ul of cDNA were supplemented with
5 ul of 10XPCR buffer, 1 ul of each dNTP (10 mM), 1 ul each
of 40 μ M primer solutions, 1.25 units of Taq polymerase,
and water, to a total volume of 50 ul. Each mixture was
heated for five minutes at 94°C. Amplification was then
carried out for 30 cycles (MAGE-1: 1 minute at 94°C, 3
minutes at 72°C; MAGE-2: 1 minute at 94°C, 2 minutes at
15 67°C; MAGE-3: 1 minute at 94°C, 4 minutes at 72°C).
Cycling was concluded, in each case, with a final extension
at 72°C for 15 minutes. A 10 ul sample of each reaction
was run on a 1% agarose gel, and visualized by ethidium
bromide fluorescence. To ensure that RNA was not degraded,
20 a PCR assay with primers specific for β -actin was carried
out, following the listed protocols, except that only 20
cycles were carried out with the annealing step at 65°C.
Data are summarized in the Table which follows:

Table 6 Expression of gene MAGE-1, 2 and 3 in lung tumors

	Proportion of positive samples		
	MAGE-1	MAGE-2	MAGE-3
Non-small cell lung cancer	16/46	16/46	14/46
squamous cell carcinoma	8/26	6/26	7/26
adenocarcinoma	8/18	9/18	7/18
large cell carcinoma	0/2	1/2	0/2
Small cell cancer	1/3	2/3	2/3
Normal lung samples	0/8	0/8	0/8

Example 46

The previous example showed how to identify expression of various MAGE genes. This example explains quantitation of the expression.

- 5 First, cDNA was synthesized in the same way described in example 1, except that the oligo dT consisted solely of dT₁₅, and the reaction mixture was preincubated at room temperature for 10 minutes to optimize annealing. Also, following the incubation, the transcriptase activity was
- 10 terminated by heating the mixture at 95°C for 15 minutes. PCR amplification was carried out, by combining 5 ul of 10x PCR buffer, 0.5 ul of a 2.5 mM dNTP mix, 0.2 µCi of α³²P-dCTP, 0.5 ul of each primer (40 µM solution), 1.25 units of Taq polymerase, and water, to a total of 50 ul. The
- 15 mixtures were chilled on ice, and then 5 ul of chilled cDNA solution (100 ng total RNA) were added thereto. The

mixture was heated to 94°C for five minutes, and 24 cycles of amplification were carried out (one minute at 94°C, three minutes at 72°C per cycle). Cycling concluded with a final extension at 72°C, for 15 minutes. A 15 ul sample of PCR product was run on an agarose gel which was then fixed in 10% trichloroacetic acid for 30 minutes, dried, and then exposed to a phospho-screen for 90 minutes before scanning by Phosphor-Imager to measure incorporated ³²P. This was compared to the incorporations from various dilutions of RNA of reference melanoma cell line MZ2-MEL-3.0.

Quantitative measurements of β-actin messenger and "GAPDH" (i.e., glyceraldehyde 3-phosphate dehydrogenase) was carried out on each cDNA sample, under similar conditions. The one difference was that only 18 amplification cycles were carried out. A separate PCR reaction was set up with primers for β-actin and GAPDH, with only β-actin used for normalization. Results were expressed via formula:

$$100 \times \frac{\left[\frac{\text{MAGE-1-S}}{\text{Actin-S}} \right]}{\left[\frac{\text{MAGE-1-MEL}}{\text{Actin-MEL}} \right]}$$

where: S = product from tumor sample
MEL = product from MZ2-MEL 3.0

The results obtained were comparable to those obtained previously with melanoma tumors. Level of expression varied, from 1 to 160% of the amount expressed by the reference cell line. Figure 2 presents some of these results (i.e., normalized results, relative to levels of β-actin expression). Values are percent of the level of MAGE-1 expression measured with RNA of the reference line

MZ2-MEL-3.0. Values are for MAGE-1 positive tumors of Table 2). Table 7, which follows, summarizes patterns of expression for various tumors.

Table 7. Pattern of expression of genes MAGE-1,2 and 3 by MAGE-positive lung tumor samples

	MAGE-1*	MAGE-2	MAGE-3
Squamous cell carcinoma			
LB 175	++	++	+++
LB 178	++	-	-
LB 182 (A1)*	-	+	-
LB 195	+	++	+++
LB 206	+++	+	++
LB 321	+	-	-
LB 323	+++	+	+++
LB 424	+	-	+
LB 425	-	-	+
LB 498 (A1)	+++	-	-
LB 557	-	+++	+++
Adenocarcinoma			
LB 117 (A1)	+	++	++
LB 212	++	+	-
LB 264 (A1)	+++	++	+++
LB 292	-	++	+++
LB 306	++	+	++
LB 322	-	+	+
LB 474 (A1)	+	++	-
LB 497	++	+++	+++
LB 510	+++	-	-
LB 558 (A1)	+	+	+
Large cell carcinoma			
LB 259	-	+	-
Small cell lung cancer			
LB 444	-	++	+++
LB 648 (A1)	+	++	+++

Example 47

The expression of the MAGE-3 gene in various tumors and normal tissues was evaluated, using both reverse transcription and polymerase chain reaction ("PCR") amplification. To perform these assays, the total RNA of the cells of interest was extracted via the well known guanidine-isothiocyanate procedure of Davis et al., Basic Methods in Molecular Biology, 1986 (New York, Elsevier, pp. 130), which is incorporated by reference in its entirety. cDNA was then synthesized, by taking 2 ug of the RNA, diluting it with water, and then adding the following materials: 4 ul of 5X reverse transcriptase buffer, 1 ul each of each dNTP (10 mM), 2 ul of a 20 μ M solution of oligo dT, 20 units of RNasin, 2 ul of 0.1 M dithiothreitol, and 200 units of MoMLV reverse transcriptase. All materials were mixed in a 20 ul reaction volume, and incubated at 42°C for 60 minutes. For the amplification reaction, 1/20 of the cDNA reaction product was supplemented with 5 ul of PCR buffer, 0.5 ul of each of the dNTPs (10 mM), 1 ul each of 20 μ M solutions of primer (see infra), and 1.25 units of Taq polymerase. Water was added to a final volume of 50 uls. The primers used for MAGE-3 were:

5'-TGGAGGACCAGAGGCCCCC-3'
(SEQ ID NO: 27)

5'-GGACGATTATCAGGAGGCCTGC-3'
(SEQ ID NO: 28)

These correspond to a sense sequence in exon 2 of the gene (SEQ ID NO: 27), and an antisense sequence in exon 3 (SEQ ID NO: 28).

PCR was performed for 30 cycles (one minute at 94°C, four minutes at 72°C). PCR products were size fractionated on a 1% agarose gel, and then analyzed. The results are presented in the table which follows. These data confirm

some results obtained previously, but also show the expression of MAGE-3 in head and neck squamous cell carcinomas, a result not suggested by previous work.

Table 8. Expression of gene MAGE-3 by tumoral, normal and fetal tissues.

TUMORS			NORMAL TISSUES	
HISTOLOGICAL TYPE	Number of MAGE-3 positive tumors* cell lines	tumors samples	HISTOLOGICAL TYPE	MAGE-3 expression*
Melanomas	50/62 (81%)	72/105 (69%)	ADULT TISSUES	
Head and neck squamous cell carcinomas	-	20/36 (56%)	Brain	-
Lung carcinomas	1/2	14/46 (30%)	Colon	-
NSCLC ‡	18/22 (82%)	2/3	Stomach	-
SCLC	5/16	5/31 (16%)	Liver	-
Colorectal carcinomas	2/6	16/132 (12%)	Ovary	-
Mammary carcinomas	-	2/6	Skin	-
Bladder tumors	1/4	3/10	Lung	-
Sarcomas	-	3/20	Kidney	-
Prostatic carcinomas	0/5	0/18	Breast	-
Renal carcinomas	2/6	0/20	Testis	++
Leukemias	0/6	0/5	FETAL TISSUES	
Lymphomas	-	-	Brain	-
			Liver	-
			Spleen	-

*Expression of gene MAGE-3 was tested by RT-PCR amplification on total RNA, with the primers described in methods. These primers distinguish MAGE-3 from the 11 other MAGE genes that have been identified.

‡ NSCLC are non-small cell lung carcinomas, SCLC are small cell lung carcinomas.

Example 48

Bladder tumor specimens were collected at surgery. They were divided into two portions, one of which was used for routine histopathological evaluation. The other
5 portion was frozen in liquid nitrogen immediately after transurethral resection, or radical cystectomy. These frozen samples were stored at -80°C until used for RNA extraction. Normal bladder tissue was obtained by biopsies of cadavers from donors in an organ transplant program.

10 Total RNA was extracted from the samples by the classic guanidine-isothiocyanate/cesium chloride method of Davis et al, Basic Methods in Molecular Biology, pp. 130-135, Elsevier, New York (1986). Synthesis of cDNA was then carried out by extension with oligo(dT) using 2 ug of RNA
15 in a 20 ul reaction volume following DeSmet et al., Immunogenetics 39: 121-129 (1994), incorporated by reference herein. Following incubation at 42°C for one hour, the cDNA reaction mixture was diluted to 100 ul with water. Separate polymerase chain reaction amplification
20 were then carried out to determine whether any of MAGE-1, 2, 3 or 4 cDNA were present. The amplifications were carried out using oligonucleotide primers located in different exons of the MAGE genes. PCR amplification was also carried out using primers for HLA-A1.

25 The primers used were the following:

5'-TGGAGGACCAGAGGCCCCC-3 (sense, exon 2) (SEQ ID NO: 27)
and

5'-GGACGATTATCAGGAGGCCTGC-3' (antisense, exon 3) (SEQ ID NO: 28) for MAGE-3

30 5'-CGGCCGAAGGAACCTGACCCAG-3' (sense, exon 1) (SEQ ID NO: 47) and

5'-GCTGGAACCCTCACTGGGTTGCC-3' (anti-sense, exon 3) (SEQ ID NO: 48) for MAGE-1

35 5'-AAGTAGGACCCGAGGCACTG-3' (sense, exon 2) (SEQ ID NO: 49)
and

5'-GAAGAGGAAGAAGCGGTCTG-3' (anti-sense, exon 3) (SEQ ID NO: 50) for MAGE-2

5'-GAGCAGACAGGCCAACCG-3' (sense, exon 2) (SEQ ID NO: 29)
and

5'-AAGGACTCTGCGTCAGGC-3' (anti-sense, exon 3) (SEQ ID NO:
30) for MAGE-4

5 5'-GGGACCAGGAGACACGGAATA-3' (sense, exon 2) (SEQ ID NO: 51)
and

5'-AGCCCGTCCACGCACCG-3' (anti-sense, exon 3) (SEQ ID NO:
52) for HLA-A1

10 SEQ ID NOS: 27 and 28 are described by Weynants et al.,
Int. J. Cancer 56: 826-829 (1994). SEQ ID NOS: 47 and 48
are described in Brasseur et al., Int. J. Cancer 52: 839-
841 (1992). SEQ ID NOS: 49 and 50 are disclosed in DeSmet
et al., Immunogenetics 39: 121-129 (1994). SEQ ID NOS: 29
and 30 are disclosed in copending application Serial No.
15 08/299,849 filed September 1, 1994 to DePlaen et al., and
incorporated by reference. SEQ ID NOS: 51 and 52 are found
in Gaugler et al., J. Exp. Med. 179: 921-930 (1994), as
well as the above-identified parent application. All of
these references are incorporated by reference.

20 The amplification protocol was as follows. Each PCR
reaction used 5 ul of cDNA, supplemented with 5 ul of 10x
PCR buffer, 1 ul each of 10 mM dNTP, 0.5 ul each of 80 uM
solutions of primers, 1.25 units of Taq DNA polymerase, and
water to achieve a total volume of 50 ul. The mixtures
25 were heated to 94°C for 5 minutes, followed by
amplification in a thermal cycler, for 30 cycles. For
MAGE-1, 1 cycle was one minute at 94°C followed by three
minutes at 72°C. For MAGE-2, one cycle was 94°C for one
minute, followed by two minutes at 67°C and two minutes at
30 72°C. For MAGE-3, one cycle was one minute at 94°C;
followed by four minutes at 72°C. For MAGE-4, one cycle
was one minute at 94°C, two minutes at 68°C, and two
minutes at 72°C. The cycle for HLA-A1 was the same as that
for MAGE-4. A 10 ul sample of each reaction was run on a
35 1% agarose gel, and then visualized by ethidium bromide
fluorescence. In order to provide a control for RNA

integrity, a 20 cycle PCR assay, using primers specific for β actin, was carried out in each case, following Weynants et al., supra.

5 The protocols described were developed with certain goals in mind. Primers were selected so as to be in different exons, thus preventing false positives due to DNA contamination of the RNA preparations. Under the conditions used, DNA generates either no PCR product, or longer products which are readily distinguishable from
10 amplified cDNA. This is shown by figure 19. In figure 19, a bladder tumor sample from a patient, referred to as "HM15" is shown in each "R" lane. Lanes marked "D" show products obtained from amplification of the patients' genomic DNA. The PCR products were run on a 2.5% low
15 melting agarose gel, but the assays were identical to the protocol of this example in all other ways. Size markers are on the left hand side. There was no band in the MAGE-1 reaction, because of the large intron between the two primers.

20 Table 10, which follows, shows the results obtained for a number of tumors (nomenclature is explained below). Of 57 samples of primary transitional cell carcinoma, 21% expressed MAGE-1, 30% expressed MAGE-2, 35% expressed MAGE-3, and 33% expressed MAGE-4. Ta tumors and low grade T1
25 tumors expressed none of these, or expressed only a single gene, at low levels. Higher stage tumors, in contrast, frequently expressed high levels of several genes. It was also found that the fraction of invasive tumors which expressed MAGE genes was 2-5 times higher than the fraction
30 observed with superficial tumors, as is depicted in figure 2 (this figure is based upon data from Table 10). Tumors expressing at least one of the four MAGE genes accounted for 61% of the 28 invasive tumors studied. Among the 29 superficial tumors, the proportion was only 28%. Results
35 paralleled other results reported previously for melanoma, in that all but one of the tumors expressing MAGE-1 also expressed MAGE-3.

None of the six biopsies of normal bladder examined expressed any of the MAGE genes discussed herein.

In some instances, several tumor samples were obtained from the same patient. The analysis of these patients is set out in Table 9. Patient HM61 had a primary tumor and an invaded lymph node. They displayed a very similar pattern of expression of MAGE-1, 2, and 3, with MAGE-1 predominating. Normal mucosa adjacent to the tumor was completely negative for MAGE-2 and MAGE-3, with a very low level of MAGE-1 expression, which was probably due to the presence of a few tumor cells. In patient "HM25", the initial tumor, and an early recurrence, both expressed MAGE-1, 2, 3 and 4. A recurrence which occurred two years after the first displayed a very different pattern, expressing only MAGE-2 and MAGE-3. A similar discordance between primary tumor and recurrence was observed with patient "HM20". Patients HM30 and LB526 showed differences in the pattern of MAGE-expression in different samples of the same primary tumor.

In the tables which follow, "Ta" stands for a superficial lesion, limited to bladder mucosa (also known as "stage Ta"). "Stage T1", or "T1" is used for superficial lesions limited to subepithelial connective tissue. "Stages T2-T4", or "T2-T4" refer to tumors which have invaded bladder muscle. The nomenclature "G1", "G2" and "G3" refers to the degree of differentiation, or histopathological grade. "G1" superficial tumor is well differentiated, while a "G3" tumor is poorly differentiated. See Mostofi et al., "Histological Typing of Urinary Bladder Tumors. WHO International Histological Classification of Tumors" (1973).

TABLE 9 - EXPRESSION OF GENES MAGE-1, 2, 3 AND 4
IN MULTIPLE SAMPLES FROM BLADDER CARCINOMA PATIENTS

Patients	Samples	Tumor stage		and grade	MAGE			
					MAGE-1	MAGE-2	MAGE-3	MAGE-4
HM 61	Primary tumor			T2 G3	++	+	+	-
	Metastatic iliac lymph node				+++	+	+	-
	Mucosa adjacent to the tumor				+	-	-	-
HM 25	Primary tumor			T2 G2	+	++	+	++
	Tumor recurrence after 1 month			T2 G2	++	++	++	++
	Tumor recurrence after 2 years			T1 G2	-	+++	+++	-
HM 20	Primary tumor			T1 G1	+	-	-	-
	Tumor recurrence after 2 months			T1 G1	-	-	-	-
HM 30	Primary tumor, 1st sample			T2 G2	-	-	++	-
	Primary tumor, 2nd sample			T2 G2	-	-	-	-
LB 526	Primary tumor, radical cystectomy			T3 G2	+	+	++	+
	Primary tumor, 9-day pre-operative biopsy			T3 G2	+	+	+	-

Table 10. EXPRESSION OF GENES MAGE-1, 2, 3 AND 4 IN BLADDER TRANSITIONAL-CELL CARCINOMA SAMPLES

Tumor Stage and Grade ‡		Patients	MAGE-1	MAGE-2	MAGE-3	MAGE-4 ¶
Superficial tumors (n=29)						
Ta (n=7)	G1	HM 7	-	-	+	-
		HM 32 (A1)°	-	-	+	-
		HM 33 (A1)	-	-	-	-
		HM 49	-	-	-	-
G2	LB 523	-	-	-	-	
	LB 817	-	-	-	-	
	LB 818	-	-	-	-	
T1 (n=22)	G1	HM 2	-	-	-	-
		HM 6 (A1)	-	-	-	-
		HM 17	-	-	-	-
		HM 20	-	-	-	-
		HM 22	-	-	-	-
		HM 34	-	-	-	-
		HM 35	-	-	-	+
		G2	HM 4	-	+	+
	HM 5		-	-	-	-
	HM 9		-	-	-	-
	HM 27		-	-	-	+++
	HM 37		-	-	-	-
	HM 38 (A1)		-	-	-	-
	HM 39		-	-	-	+
	G3	HM 40 (A1)	-	-	-	-
		HM 41	-	-	-	-
		HM 14	++	+++	+++	++
Invasive tumors (n=28)	T2 (n=15)	HM 23	-	+++	+++	-
		HM 26	-	+++	+++	+++
		HM 42 (A1)	-	-	+++	+++
		HM 53	-	-	-	-
		LB 767 (A1)	-	-	-	-
T3 (n=11)	G2	HM 8	-	-	-	-
		HM 13 (A1)	-	-	-	-
		HM 24 (A1)	+	+++	+++	++
		HM 25	+	++	+	++
		HM 30	-	-	+	-
		LB 796	-	-	+	-
	G3	HM 3 (A1)	-	-	-	-
		HM 10	-	+	+	++
		HM 12	-	+	+	-
		HM 15	+++	+++	+++	+++
		HM 61 (A1)	++	+	+	+
		LB 524 (A1)	-	-	+++	+
		LB 824	-	++	+++	+
		LB 825	+	++	+++	+
	G2	LB 831	+	++	+++	++
		HM 44	-	-	-	-
		HM 45	-	+++	+++	-
HM 46 (A1)		-	-	+	-	
LB 526		+	+	++	+	
G3	HM 11	++	+	++	+	
	HM 18	-	-	-	-	
	HM 21	-	-	-	-	
	HM 47	+++	+++	+++	+++	
	HM 48	-	-	-	-	
	HM 50	+++	+++	+++	+	
	HM 52 (A1)	-	-	-	-	
T4 (n=2)	G3	HM 1	-	-	-	+
		HM 51	-	-	-	-

The foregoing examples show that expression of MAGE tumor rejection antigen precursors is correlated to various cancers. One aspect of the invention, then, is a method for determining these cancers by assaying a sample for expression of at least one MAGE tumor rejection antigen precursor. As MAGE genes are nearly without exception expressed only by tumor cells, there can be no question but that expression of a MAGE gene or genes is indicative of cancer. The fact that the cancer is a particular type, such as lung adenocarcinoma, is easily ascertainable, as adenocarcinoma cells have distinct morphologies which are identifiable by the skilled artisan. Similarly, the fact that the tumor of interest is a lung adenocarcinoma as compared to a tumor from a different body part is self evident; one does not find lung adenocarcinoma in, e.g., large intestine tissue. Analogous statements can be made for bladder and other cancers.

The assay for the MAGE genes can take many forms. Most preferably, the assay is done via determining gene expression, such as by determining mRNA transcription products. For example, amplification protocols, including but not being limited to polymerase chain reaction (PCR), and ligase chain reaction (LCR), are preferred. The assay can also be carried out using nucleic acid molecule probes, which are labelled or unlabelled, and which specifically hybridize to sequences characteristic of the MAGE gene of interest. Labelling nucleotide probes is well known to the art, labels including radioactive, fluorescent, chromophoric, magnetic, and other identifiable materials. Antibodies, haptens such as biotin, (strept)avidin, digoxin, digoxigenin, and so forth, can all be used. Non-labelled probes can also be used. In such a case, the probes will form a double stranded molecule with their target. Any remaining single stranded material can be enzymatically digested, and when something remains, it is a sign of MAGE expression. For the case of polymerase chain reaction or other methodologies where a primer or

primers are required, the molecules represented by SEQ ID NO: 47 and SEQ ID NO: 48 are especially preferred for MAGE-1, SEQ ID NO: 49 and 50 for MAGE-2, SEQ ID NOS: 27 and 28 for MAGE-3 and SEQ ID NOS: 29 and 30 for MAGE-4.

5 Similarly, these molecules are preferred as probes.

Quantitation of MAGE expression is shown herein as well. This is an important feature of the invention because in a given tumor sample (as compared to tumor cell lines) there will always be an undetermined proportion of
10 normal cells.

One may also assay for the expression product of the MAGE gene, e.g., the tumor rejection antigen precursor protein, via assays such as immunoassays. See, e.g., U.S. Patent Application Serial No. 08/190,411 filed February 1, 1994, and Chen, et al., Proc. Natl. Acad. Sci. USA 91(3):
15 1004-1008 (1994), both of which are incorporated by reference, teaching MAGE-1 specific mAbs.

The terms and expressions which have been employed are used as terms of description and not of limitation, and
20 there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

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- (ii) TITLE OF INVENTION: Method For Determining A Cancerous Condition by Assaying For Expression Of One Or More Mage Tumor Rejection Antigen Precursors
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66

- (2) INFORMATION FOR SEQUENCE ID NO: 1:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 462 base pairs
 (B) TYPE: nucleic acid
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: genomic DNA
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ACCACAGGAG	AATGAAAAGA	ACCCGGGACT	CCCAAAGACG	CTAGATGTGT	50
GAAGATCCTG	ATCACTCATT	GGGTGTCTGA	GTTCTGCGAT	ATTCATCCCT	100
CAGCCAATGA	GCTTACTGTT	CTCGTGGGGG	GTTTGTGAGC	CTTGGGTAGG	150
AAGTTTGTGA	AGTTCCGCCT	ACAGCTCTAG	CTTGTGAATT	TGTACCCTTT	200
CACGTAAAAA	AGTAGTCCAG	AGTTTACTAC	ACCCTCCCTC	CCCCCTCCCA	250
CCTCGTGCTG	TGCTGAGTTT	AGAAGTCTTC	CTTATAGAAG	TCTTCGGTAT	300
AGAACTCTTC	CGGAGGAAGG	AGGGAGGACC	CCCCCCTTTT	GCTCTCCAG	350
CATGCATTGT	GTCAACGCCA	TTGCACTGAG	CTGGTCGAAG	AAGTAAGCCG	400
CTAGCTTGCG	ACTCTACTCT	TATCTTAACT	TAGCTCGGCT	TCCTGCTGGT	450
ACCCTTTGTG	CC				462

- (2) INFORMATION FOR SEQUENCE ID NO: 2:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 675 base pairs
 (B) TYPE: nucleic acid
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: genomic DNA
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA GGT GGT	48
Met Ser Asp Asn Lys Lys Pro Asp Lys Ala His Ser Gly Ser Gly Gly	
5 10 15	
GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG TAC TCC CTG GAA	96
Asp Gly Asp Gly Asn Arg Cys Asn Leu His Arg Tyr Ser Leu Glu	
20 25 30	
GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC TTC GCT GTT GTC ACA ACA	144
Glu Ile Leu Pro Tyr Leu Gly Trp Leu Val Phe Ala Val Thr Thr	
35 40 45	
AGT TTT CTG GCG CTC CAG ATG TTC ATA GAC GCC CTT TAT GAG GAG CAG	192
Ser Phe Leu Ala Leu Gln Met Phe Ile Asp Ala Leu Tyr Glu Glu Gln	
50 55 60	
TAT GAA AGG GAT GTG GCC TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC	240
Tyr Glu Arg Asp Val Ala Trp Ile Ala Arg Gln Ser Lys Arg Met Ser	
65 70 75 80	
TCT GTC GAT GAG GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC	288
Ser Val Asp Glu Asp Glu Asp Asp Glu Asp Asp Glu Asp Asp Tyr Tyr	
85 90 95	
GAC GAC GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT	336
Asp Asp Glu Asp Asp Asp Asp Ala Phe Tyr Asp Asp Glu Asp Asp	
100 105 110	
GAG GAA GAA GAG TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA GAT GAG	384
Glu Glu Glu Glu Leu Glu Asn Leu Met Asp Asp Glu Ser Glu Asp Glu	
115 120 125	
GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA GCT GAG GAA ATG	432
Ala Glu Glu Glu Met Ser Val Glu Met Gly Ala Gly Ala Glu Glu Met	
130 135 140	
GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT GGC CAT CAT TTA AGG AAG	480
Gly Ala Gly Ala Asn Cys Ala Cys Val Pro Gly His His Leu Arg Lys	
145 150 155 160	
AAT GAA GTG AAG TGT AGG ATG ATT TAT TTC TTC CAC GAC CCT AAT TTC	528
Asn Glu Val Lys Cys Arg Met Ile Tyr Phe Phe His Asp Pro Asn Phe	
165 170 175	
CTG GTG TCT ATA CCA GTG AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT	576
Leu Val Ser Ile Pro Val Asn Pro Lys Glu Gln Met Glu Cys Arg Cys	
180 185 190	

67

GAA AAT GCT GAT GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAA GAG	624
Glu Asn Ala Asp Glu Glu Val Ala Met Glu Glu Glu Glu Glu Glu Glu	
195 200 210	
GAG GAG GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC TTC TCA CCT	672
Glu Glu Glu Glu Glu Glu Glu Met Gly Asn Pro Asp Gly Phe Ser Pro	
220 225 230 235	
TAG	675

- (2) INFORMATION FOR SEQUENCE ID NO: 3:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 228 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCATGCAGTT GCAAAGCCCA GAAGAAAGAA ATGGACAGCG GAAGAAGTGG TTGTTTTTTT	60
TTCCCCTTCA TTAATTTTCT AGTTTTTAGT AATCCAGAAA ATTTGATTTT GTTCTAAAGT	120
TCATTATGCA AAGATGTCAC CAACAGACTT CTGACTGCAT GGTGAACCTT CATATGATAC	180
ATAGGATTAC ACTTGACCT GTTAAAAATA AAAGTTTGAC TTGCATAC	228

- (2) INFORMATION FOR SEQUENCE ID NO: 4:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1365 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ACCACAGGAG AATGAAAAGA ACCCGGGACT CCCAAAGACG CTAGATGTGT	50
GAAGATCCTG ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT	100
CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG	150
AAGTTTTGCA AGTTCCGCCT ACAGCTCTAG CTTGTGAATT TGTACCCCTT	200
CACGTAAGAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA	250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT	300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCTTT GCTCTCCAG	350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG	400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT	450
ACCCTTTGTG CC	462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA	504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG	546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC	588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC	630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC	672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG	714
GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC GAC GAC	756
GAG GAC GAC GAC GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT	798
GAG GAA GAA GAA TTT GAG AAC CTG ATG GAT GAT GAA TCA GAA	840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA	882
GCT GAG GAA ATG GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT	924
GGC CAT CAT TTA AGG AAG AAT GAA GTG AAG TGT AGG ATG ATT	966
TAT TTC TTC CAC GAC CCT AAT TTC CTG GTG TCT ATA CCA GTG	1008
AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT GAA AAT GCT GAT	1050
GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAG GAG GAG	1092
GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC TTC TCA CCT	1134
TAG	1137
GCATGCAGTT GCAAAGCCCA GAAGAAAGAA ATGGACAGCG GAAGAAGTGG	1187
TTGTTTTTTT TTCCCCTTCA TTAATTTTCT AGTTTTTAGT AATCCAGAAA	1237
ATTTGATTTT GTTCTAAAGT TCATTATGCA AAGATGTCAC CAACAGACTT	1287
CTGACTGCAT GGTGAACCTT CATATGATAC ATAGGATTAC ACTTGACCTT	1337
GTAAAAATA AAAGTTTGAC TTGCATAC	1365

(2) INFORMATION FOR SEQUENCE ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4698 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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ACCACAGGAG AATGAAAAGA ACCCGGGACT CCCAAAGACG CTAGATGTGT      50
GAAGATCCTG ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT      100
CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTGTGAGC CTTGGGTAGG      150
AAGTTTTGCA AGTCCGCCT ACAGCTCTAG CTTGTGAATT TGTACCCCTT      200
CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA      250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT      300
AGAACTCTTC CGAGGAAGG AGGGAGGACC CCCCCCTTTT GCTCTCCAG      350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG      400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT      450
ACCCCTTGTG CC                                     462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA      504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG      546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC      588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC      630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC      672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG      714
GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC GAC GAC      756
GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT      798
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA      840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA      882
GCT GAG GAA ATG GGT GCT GGC GCT AAC TGT GCC T      916
GTGAGTAACC CGTGGTCTTT ACTCTAGATT CAGGTGGGGT GCATTCTTTA      966
CTCTTGCCCA CATCTGTAGT AAAGACCACA TTTTGGTTGG GGGTCATTGC      1016
TGGAGCCATT CCTGGCTCTC CTGTCCACGC CTATCCCCGC TCCTCCCATC      1066
CCCCACTCCT TGCTCCGCTC TCTTTCCTTT TCCACCTTG CCTCTGGAGC      1116
TTCAGTCCAT CCTGCTCTGC TCCCTTTCCC CTTGCTCTC CTTGCTCCCC      1166
TCCCCCTCGG CTCAACTTTT CGTGCCTTCT GCTCTCTGAT CCCCACCCTC      1216
TTCAGGCTTC CCATTGCTCT CCTCTCCCGA AACCCCTCCC TTCCTGTTCC      1266
CCTTTTCGCG CCTTTTCTTT CCTGCTCCCC TCCCCCTCCC TATTTACCTT      1316
TCACCGCTT TGCTCTCCCT GCTCCCTCC CCCTTTTGCA CCTTTTCTTT      1366
TCCTGCTCCC CTCCCCCTCC CCTCCCTGTT TACCCTTCAC CGCTTTTCTT      1416
CTACCTGCTT CCCTCCCCCT TGCTGCTCCC TCCTATTTTG CATTTTCGGG      1466
TGCTCCTCCC TCCCCCTCCC CCTCCCTCCC TATTTGCATT TTCGGGTGCT      1516
CCTCCCTCCC CCTCCCCAGG CCTTTTTTTT TTTTTTTTTT TTTTTTTTTT      1566
TTGGTTTTTC GAGACAGGGT TTCTCTTTGT ATCCCTGGCT GTCCTGGCAC      1616
TCACTCTGTA GACCAGGCTG GCCTCAAAC CAGAAATCTG CCTGCCTCTG      1666
CCTCCCAAT GCTGGGATTA AAGGCTTGCA CCAGGACTGC CCCAGTGCAG      1716
GCCTTTCTTT TTTCTCTCT CTGGTCTCCC TAATCCCTTT TCTGCATGTT      1766
AACTCCCTTT TTGGCACCTT TCCTTTACAG GACCCCTCC CCCTCCCTGT      1816
TTCCCTTCCG GCACCCTTCC TAGCCCTGCT CTGTTCCCTC TCCCTGCTCC      1866
CCTCCCCCTC TTTGCTCGAC TTTTAGCAGC CTTACCTCTC CCTGCTTTCT      1916
GCCCGGTTCC CCTTTTTTGT GCCTTTCCTC CTGGCTCCCC TCCACCTTCC      1966
AGCTCACCTT TTTGTTTGTG TGGTTGTTTG GTTGTGTTGG TTGCTTTTTT      2016
TTTTTTTTTT GCACCTTGTT TTCCAAGATC CCCCTCCCCC TCCGGCTTCC      2066
CCTCTGTGTG CCTTTCCTGT TCCCTCCCCC TCGCTGGCTC CCCCTCCCTT      2116
TCTGCCTTTC CTGTCCCTGC TCCCTTCTCT GCTAACCTTT TAATGCCCTT      2166
CTTTTCTAGA CTCCCCCTC CAGGCTTGCT GTTTGCTTCT GTGCACTTTT      2216
CCTGACCCTG CTCCCCCTCC CCTCCAGCT CCCCCCTCTT TTCCACCTC      2266
CCTTCTCCA GCCTGTCACC CCTCCTCTC TCCTCTCTGT TTCTCCACT      2316
TCCTGCTTCC TTTACCCTTT CCCTCTCCCT ACTCTCCTCC CTGCCTGCTG      2366
GACTTCTCT CCAGCGGCC AGTTCCTGCG AGTCCTGGAG TCTTTCCTGC      2416
CTCTCTGTCC ATCACTTCCC CCTAGTTTCA CTTCCCTTTC ACTCTCCCT      2466
ATGTGTCTCT CTTCTATCT ATCCCTTCTT TTCTGTCCCC TCTCCTCTGT      2516
CCATCACCTC TCTCTCCCT TCCCTTTCCT CTCTCTTCCA TTTTCTTCCA      2566
CCTGCTTCTT TACCCTGCCT CTCCATTGC CCTTTACCT TTATGCCCAT      2616
TCCATGTCCC CTCTCAATTC CCTGTCCCAT TGTGCTCCCT CACATCTTCC      2666
ATTTCCCTCT TTCTCCCTTA GCCTCTTCTT CCTCTTCTCT TGTATCTCCC      2716

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TTCCCTTTGC	TTCTCCCTCC	TCCTTTCCCC	TTCCCTTATG	CCCTCTACTC	2766
TACTTGATCT	TCTCTCCTCT	CCACATACCC	TTTTTCCTTT	CCACCCTGCC	2816
CTTTGTCCCC	AGACCCTACA	GTATCCTGTG	CACAGGAAGT	GGGAGGTGCC	2866
ATCAACAACA	AGGAGGCAAG	AAACAGAGCA	AAATCCCAAA	ATCAGCAGGA	2916
AAGGCTGGAT	GAAAAAAGG	CCAGGTTCTG	AGGACAGCTG	GAATCTAGCC	2966
AAGTGGCTCC	TATAACCCTA	AGTACCAAGG	GAGAAAGTGA	TGGTGAAGTT	3016
CTTGATCCTT	GCTGCTTCTT	TTACATATGT	TGGCACATCT	TTCTCAAATG	3066
CAGGCCATGC	TCCATGCTTG	GCGCTTGCTC	AGCGTGGTTA	AGTAATGGGA	3116
GAATCTGAAA	ACTAGGGGCC	AGTGGTTTGT	TTTGGGGACA	AATTAGCAGC	3166
TAGTGATATT	TCCCCCTAAA	AATTATAACA	AACAGATTCA	TGATTTGAGA	3216
TCCTTCTACA	GGTGAGAAGT	GGAAAAATTG	TCACTATGAA	GTTCTTTTAA	3266
GGCTAAAGAT	ACTTGAACC	ATAGAAGCGT	TGTTAAATA	CTGCTTTCTT	3316
TTGCTAAAAT	ATTCTTTCTC	ACATATTCAT	ATTCTCCAG		3355
GT GTT CCT	GGC CAT CAT	TTA AGG AAG	AAT GAA	GTG AAG TGT	3396
AGG ATG ATT	TAT TTC TTC	CAC GAC CCT	AAT TTC	CTG GTG TCT	3438
ATA CCA GTG	AAC CCT AAG	GAA CAA ATG	GAG TGT	AGG TGT GAA	3480
AAT GCT GAT	GAA GAG GTT	GCA ATG GAA	GAG GAA	GAA GAA GAA	3522
GAG GAG GAG	GAG GAG GAA	GAG GAA ATG	GGA AAC	CCG GAT GGC	3564
TTC TCA CCT	TAG				3576
GCATGCAGGT	ACTGGCTTCA	CTAACCAACC	ATTCTTAACA	TATGCCTGTA	3626
GCTAAGAGCA	TCTTTTAAAA	AAATATTATT	GGTAAACTAA	ACAATTGTAA	3676
TCTTTTACA	TTAATAAGTA	TTAAATTAAT	CCAGTATACA	GTTTTAAGAA	3726
CCCTAAGTTA	AACAGAAGTC	AATGATGTCT	AGATGCCTGT	TCTTTAGATT	3776
GTAGTGAGAC	TACTTACTAC	AGATGAGAAG	TTGTTAGACT	CGGGAGTAGA	3826
GACCACTAAA	AGATCATGCA	GTGAAATGTG	GCCATGGAAA	TCGCATATTG	3876
TTCTTATAGT	ACCTTTGAGA	CAGCTGATAA	CAGCTGACAA	AAATAAGTGT	3926
TTCAAGAAAG	ATCACACGCC	ATGGTTCACA	TGCAAATTAT	TATTTTGTCTG	3976
TTCTGATTTT	TTTCATTCT	AGACCTGTGG	TTTTAAAGAG	ATGAAAATCT	4026
CTTAAATTTT	CCTTCATCTT	TAATTTTCCT	TAACTTTAGT	TTTTTTCACT	4076
TAGAATTCAA	TTCAAATTCT	TAATTCAATC	TTAATTTTAA	GATTTCTTAA	4126
AATGTTTTTT	AAAAAAAATG	CAAAATCTCAT	TTTTAAGAGA	TGAAAGCAGA	4176
GTAAGTGGG	GGCTTAGGGA	ATCTGTAGGG	TTGCGGTATA	GCAATAGGGA	4226
GTTCTGGTCT	CTGAGAAGCA	GTCAGAGAGA	ATGGAAAACC	AGGCCCTTGC	4276
CAGTAGGTTA	GTGAGGTGTA	TATGATCAGA	TTATGGACAC	TCTCAAATC	4326
ATAAATACTC	TAACAGCTAA	GGATCTCTGA	GGGAAACACA	ACAGGGAAT	4376
ATTTTAGTTT	CTCCTTGAGA	AACAATGACA	AGACATAAAA	TTGGCAAGAA	4426
AGTCAGGAGT	GTATTCTAAT	AAGTGTGCT	TATCTCTTAT	TTTCTTCTAC	4476
AGTTGCAAG	CCCAGAAGAA	AGAAATGGAC	AGCGGAAGAA	GTGGTTGTTT	4526
TTTTTTCCCC	TTCATTAAAT	TTCTAGTTT	TAGTAATCCA	GAAAATTTGA	4576
TTTTGTTCTA	AAGTTTCTTA	TGCAAAGATG	TCACCAACAG	ACTTCTGACT	4626
GCATGGTGAA	CTTTCATATG	ATACATAGGA	TTACACTTGT	ACCTGTTAAA	4676
AATAAAAGTT	TGACTTGCAT	AC			4698

- (2) INFORMATION FOR SEQUENCE ID NO: 6:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Leu Pro Tyr Leu Gly Trp Leu Val Phe
5

- (2) INFORMATION FOR SEQUENCE ID NO: 7:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2418 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: genomic DNA
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGATCCAGGC	CCTGCCAGGA	AAAATATAAG	GGCCCTGCGT	GAGAACAGAG	50
GGGGTCATCC	ACTGCATGAG	AGTGGGGATG	TCACAGAGTC	CAGCCCACCC	100

TCCTGGTAGC	ACTGAGAAGC	CAGGGCTGTG	CTTGCGGTCT	GCACCCTGAG	150
GGCCCGTGGA	TTCCTCTTCC	TGGAGCTCCA	GGAACCAGGC	AGTGAGGCCT	200
TGGTCTGAGA	CAGTATCCTC	AGGTCACAGA	GCAGAGGATG	CACAGGGTGT	250
GCCAGCAGTG	AATGTTTGCC	CTGAATGCAC	ACCAAGGGCC	CCACCTGCCA	300
CAGGACACAT	AGGACTCCAC	AGAGTCTGGC	CTCACCTCCC	TACTGTCACT	350
CCTGTAGAAT	CGACCTCTGC	TGGCCGGCTG	TACCCTGAGT	ACCCTCTCAC	400
TTCCTCCTTC	AGGTTTTCAG	GGGACAGGCC	AACCCAGAGG	ACAGGATTCC	450
CTGGAGGCCA	CAGAGGAGCA	CCAGGAGAA	GATCTGTAAG	TAGGCCTTTG	500
TTAGAGTCTC	CAAGGTTTCC	TTCTCAGCTG	AGGCCTCTCA	CACACTCCCT	550
CTCTCCCTAG	GCCTGTGGGT	CTTCATTGCC	CAGCTCCTGC	CCACACTCCT	600
GCCTGCTGCC	CTGACGAGAG	TCATCATGTC	TCTTGAGCAG	AGGAGTCTGC	650
ACTGCAAGCC	TGAGGAAGCC	CTTGAGGCC	AACAAGAGGC	CCTGGGCTG	700
GTGTGTGTGC	AGGCTGCCAC	CTCCTCCTCC	TCTCCTCTGG	TCCTGGGCAC	750
CCTGGAGGAG	GTGCCCATG	CTGGGTCAAC	AGATCCTCCC	CAGAGTCCCT	800
AGGGAGCCTC	CGCCTTTCCC	ACTACCATCA	ACTTCACTCG	ACAGAGGCAA	850
CCCAGTGAGG	GTCCAGCAG	CCGTGAAGAG	GAGGGGCCAA	GCACCTCTTG	900
TATCCTGGAG	TCCTTGTTCC	GAGCAGTAAT	CACTAAGAAG	GTGGCTGATT	950
TGGTTGGTTT	TCTGCTCCTC	AAATATCGAG	CCAGGGAGCC	AGTCACAAAG	1000
GCAGAAATGC	TGGAGAGTGT	CATCAAAAAT	TACAAGCACT	GTTTTCTGTA	1050
GATCTTCGGC	AAAGCCTCTG	AGTCCTTGCA	GCTGGTCTTT	GGCATTGACG	1100
TGAAGGAAGC	AGACCCACC	GGCCACTCCT	ATGTCCTTGT	CACCTGCCTA	1150
GGTCTCTCCT	ATGATGGCCT	GCTGGGTGAT	AATCAGATCA	TGCCCCAAGC	1200
AGGCTTCTCG	ATAATTCTCC	TGGTCATGAT	TGCAATGGAG	GGCGGCCATG	1250
CTCCTGAGGA	GGAAATCTGG	GAGGAGCTGA	GTGTGATGGA	GGTGTATGAT	1300
GGGAGGGAGC	ACAGTGCCTA	TGGGGAGCCC	AGGAAGCTGC	TCACCCAAGA	1350
TTTGGTGCAG	GAAAAGTACC	TGGAGTACGG	CAGGTGCCGG	ACAGTGATCC	1400
CGCACGCTAT	GAGTTCCTGT	GGGGTCCAAG	GGCCCTCGCT	GAAACCAGCT	1450
ATGTGAAAGT	CCTTGAGTAT	GTGATCAAGG	TCAGTGCAAG	AGTTCGCTTT	1500
TTCTTCCCCT	CCCTGCGTGA	AGCAGCTTTG	AGAGAGGAGG	AAGAGGGAGT	1550
CTGAGCATGA	GTTGCAGCCA	AGGCCAGTGG	GAGGGGGACT	GGGCCAGTGC	1600
ACCTTCCAGG	GCCGCGTCCA	GCAGCTTCCC	CTGCCTCGTG	TGACATGAGG	1650
CCCATTCTTC	ACTCTGAAGA	GAGCGGTCAG	TGTTCTCAGT	AGTAGGTTTC	1700
TGTTCTATTG	GGTGAATTGG	AGATTTATCT	TTGTTCTCTT	TTGGAATTGT	1750
TCAAATGTTT	TTTTTTAAGG	GATGGTTGAA	TGAACCTCAG	CATCCAAGTT	1800
TATGAATGAT	AGCAGTCACA	CAGTCTGTG	TATATAGTTT	AAGGGTAAGA	1850
GTCTTGTTT	TTATTCAGAT	TGGGAAATCC	ATTCTATTTT	GTGAATTGGG	1900
ATAATAACAG	CAGTGGGAATA	AGTACTTAGA	AATGTGAAAA	ATGAGCAGTA	1950
AAATAGATGA	GATAAAGAAC	TAAAGAAATT	AAGAGATAGT	CAATTCCTGC	2000
CTTATACCTC	AGTCTATTCT	GTAAAATTTT	TAAAGATATA	TGCATACCTG	2050
GATTTCCCTG	GCTTCTTTGA	GAAATGTAAGA	GAAATTAAAT	CTGAATAAAG	2100
AATTCTTCCT	GTTCACTGGC	TCTTTCTTTC	TCCATGCACT	GAGCATCTGC	2150
TTTTTGGGAG	GCCTTGGGTT	AGTAGTGGAG	ATGCTAAGGT	AAGCCAGACT	2200
CATACCCACC	CATAGGGTCG	TAGAGTCTAG	GAGCTGCAGT	CACGTAATCG	2250
AGGTGGCAAG	ATGTCCTCTA	AAGATGTAGG	GAAAAGTGAG	AGAGGGGTGA	2300
GGGTGTGGGG	CTCCGGGTGA	GAGTGGTGGA	GTGTCAATGC	CCTGAGCTGG	2350
GGCATTTTGG	GCTTGGGGA	ACTGCAGTTC	CTTCTGGGGG	AGCTGATTGT	2400
AATGATCTTG	GGTGGATCC				2418

- (2) INFORMATION FOR SEQUENCE ID NO: 8:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5724 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: genomic DNA
 - (ix) FEATURE:
 - (A) NAME/KEY: MAGE-1 gene
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CCCCGGGCAC	CACTGGCATC	CCTCCCCCTA	CCACCCCAAA	TCCCTCCCTT	50
TACGCCACCC	ATCCAAACAT	CTTCACGCTC	ACCCCCAGCC	CAAGCCAGGC	100
AGAATCCGGT	TCCACCCCTG	CTCTCAACCC	AGGGAAGCCC	AGGTGCCCG	150
ATGTGACGCC	ACTGACTTGA	GCATTAGTGG	TTAGAGAGAA	GCGAGGTTTT	200
CGGTCTGAGG	GGCGGCTTGA	GATCGGTGGA	GGGAAGCGGG	CCCAGCTCTG	250

TAAGGAGGCA	AGGTGACATG	CTGAGGGAGG	ACTGAGGACC	CACTTACCCC	300
AGATAGAGGA	CCCCAAATAA	TCCCTTCATG	CCAGTCCTGG	ACCATCTGGT	350
GGTGGACTTC	TCAGGCTGGG	CCACCCCCAG	CCCCCTTGCT	GCTTAAACCA	400
CTGGGGACTC	GAAGTCAGAG	CTCCGTGTGA	TCAGGGAAGG	GCTGCTTAGG	450
AGAGGGCAGC	GTCCAGGCTC	TGCCAGACAT	CATGCTCAGG	ATTCTCAAGG	500
AGGGCTGAGG	GTCCCTAAGA	CCCCACTCCC	GTGACCCAAC	CCCCACTCCA	550
ATGCTCACTC	CCGTGACCCA	ACCCCTCTTT	CATTGTCTAT	CCAACCCCCA	600
CCCCACATCC	CCCACCCCAT	CCCTCAACCC	TGATGCCCCAT	CCGCCCAGCC	650
ATTCCACCCT	CACCCCCACC	CCCACCCCCA	CGCCCACTCC	CACCCCCACC	700
CAGGCAGGAT	CCGGTTCCCG	CCAGGAAACA	TCCGGGTGCC	CGGATGTGAC	750
GCCACTGACT	TGCGCATTGT	GGGGCAGAGA	GAAGCGAGGT	TTCCATTCTG	800
AGGGACGGCG	TAGAGTTCGG	CCGAAGGAAC	CTGACCCAGG	CTCTGTGAGG	850
AGGCAAGGTG	AGAGGCTGAG	GGAGGACTGA	GGACCCCGCC	ACTCCAAATA	900
GAGAGCCCCA	AATATTCCAG	CCCCGCCCTT	GCTGCCAGCC	CTGGCCCAAC	950
CGCGGGAAGA	CGTCTCAGCC	TGGGTGCCCC	CCAGACCCCT	GCTCCAAAAG	1000
CCTTGAGAGA	CACCAGGTTT	TTCTCCCCAA	GCTCTGGAAT	CAGAGGTTGC	1050
TGTGACCAGG	GCAGGACTGG	TTAGGAGAGG	GCAGGGCACA	GGCTCTGCCA	1100
GGCATCAAGA	TCAGCACCCA	AGAGGGAGGG	CTGTGGGCCC	CCAAGACTGC	1150
ACTCCAATCC	CCACTCCCAC	CCCATTGCGA	TTCCCATTTCC	CCACCCAACC	1200
CCCATCTCCT	CAGCTACACC	TCCACCCCCA	TCCCTACTCC	TACTCCGTCA	1250
CCTGACCACC	ACCCTCCAGC	CCCAGCACCA	GCCCCAACCC	TTCTGCCACC	1300
TCACCCTCAC	TGCCCCCAAC	CCCACCCTCA	TCTCTCTCAT	GTGCCCCACT	1350
CCCATCGCCT	CCCCCATTTCT	GGCAGAATCC	GGTTTGCCCC	TGCTCTCAAC	1400
CCAGGGAAGC	CCTGGTAGGC	CCGATGTGAA	ACCACTGACT	TGAACCTCAC	1450
AGATCTGAGA	GAAGCCAGGT	TCATTTAATG	GTTCTGAGGG	GCGGCTTGAG	1500
ATCCACTGAG	GGGAGTGGTT	TTAGGCTCTG	TGAGGAGGCA	AGGTGAGATG	1550
CTGAGGGAGG	ACTGAGGAGG	CACACACCCC	AGGTAGATGG	CCCCAAAATG	1600
ATCCAGTACC	ACCCCTGCTG	CCAGCCCTGG	ACCACCCGGC	CAGGACAGAT	1650
GTCTCAGCTG	GACCACCCCC	CGTCCCGTCC	CACTGCCACT	TAACCCACAG	1700
GGCAATCTGT	AGTCATAGCT	TATGTGACCG	GGGCAGGGTT	GGTCAGGAGA	1750
GGCAGGGCCC	AGGCATCAAG	GTCCAGCATC	CGCCCGGCAT	TAGGGTCAGG	1800
ACCCTGGGAG	GGAACTGAGG	GTTCGCCACC	CACACCTGTC	TCCTCATCTC	1850
CACCGCCACC	CCACTCACAT	TCCCATACCT	ACCCCTTACC	CCCAACCTCA	1900
TCTTGTCAGA	ATCCCTGCTG	TCAACCCACG	GAAGCCACGG	GAATGGCGGC	1950
CAGGCACTCG	GATCTTGACG	TCCCCATCCA	GGGTCTGATG	GAGGGAAGGG	2000
GCTTGAACAG	GGCCTCAGGG	GAGCAGAGGG	AGGGCCCTAC	TGCGAGATGA	2050
GGGAGGCCTC	AGAGGACCCA	GCACCCTAGG	ACACCGCACC	CCTGTCTGAG	2100
ACTGAGGCTG	CCACTTCTGG	CCTCAAGAAT	CAGAACGATG	GGGACTCAGA	2150
TTGCATGGGG	GTGGGACCCA	GGCCTGCAAG	GCTTACGCGG	AGGAAGAGGA	2200
GGGAGGACTC	AGGGGACCTT	GGAAATCCAGA	TCAGTGTGGA	CCTCGGCCCT	2250
GAGAGGTCCA	GGGCACGGTG	GCCACATATG	GCCCCATATT	CCTGCATCTT	2300
TGAGGTGACA	GGACAGAGCT	GTGGTCTGAG	AAGTGGGGCC	TCAGGTCAAC	2350
AGAGGGAGGA	GTTCCAGGAT	CCATATGGCC	CAAGATGTGC	CCCCTTCATG	2400
AGGACTGGGG	ATATCCCGG	CTCAGAAAGA	AGGGACTCCA	CACAGTCTGG	2450
CTGTCCCTTT	TTAGTAGCTC	TAGGGGGACC	AGATCAGGGA	TGGCGGTATG	2500
TTCCATTCTC	ACTTGTAACA	CAGGCAGGAA	GTTGGGGGGC	CCTCAGGGAG	2550
ATGGGCTCTT	GGGGTAAAGG	GGGGATGTCT	ACTCATGTCA	GGGAATTGGG	2600
GGTTGAGGAA	GCACAGGCGC	TGGCAGGAAT	AAAGATGAGT	GAGACAGACA	2650
AGGCTATTGG	AATCCACACC	CCAGAACCAC	AGGGGTCAGC	CCTGGACACC	2700
TCACCCAGGA	TGTGGCTTCT	TTTTCACTCC	TGTTTCCAGA	TCTGGGGCAG	2750
GTGAGGACCT	CATTCTCAGA	GGGTGACTCA	GGTCAACGTA	GGGACCCCCA	2800
TCTGGTCTAA	AGACAGAGCG	GTCCCAGGAT	CTGCCATGCG	TTCGGGTGAG	2850
GAACATGAGG	GAGGACTGAG	GGTACCCACG	GACCAGAACA	CTGAGGGAGA	2900
CTGCACAGAA	ATCAGCCCTG	CCCCTGCTGT	CACCCACAGG	AGCATGGGCT	2950
GGGCCGTCTG	CCGAGGTCCT	TCCGTTATCC	TGGGATCATT	GATGTACGGG	3000
ACGGGGAGGC	CTTGGTCTGA	GAAGGCTGCG	CTCAGGTCAG	TAGAGGGAGC	3050
GTCCCAGGCC	CTGCCAGGAG	TCAAGGTGAG	GACCAAGCGG	GCACCTCACC	3150
CAGGACACAT	TAATTCCAAT	GAATTTTGAT	ATCTCTTGCT	GCCCTTCCCC	3200
AAGGACCTAG	GCACGTGTGG	CCAGATGTTT	GTCCCTCTCT	GTCCTTCCAT	3250
TCCTTATCAT	GGATGTGAAC	TCTTGATTTG	GATTTCTCAG	ACCAGCAAAA	3300
GGGCAGGATC	CAGGCCCTGC	CAGGAAAAAT	ATAAGGGCCC	TGCGTGAGAA	3350
CAGAGGGGGT	CATCCACTGC	ATGAGAGTGG	GGATGTCACA	GAGTCCAGCC	3400
CACCCCTCCTG	GTAGCACTGA	GAAGCCAGGG	CTGTGCTTGC	GGTCTGCACC	3450
CTGAGGGCCC	GTGGATTCTT	CTTCCTGGAG	CTCCAGGAAC	CAGGCAGTGA	3500
GGCCTTGGTC	TGAGACAGTA	TCCTCAGGTC	ACAGAGCAGA	GGATGCACAG	3550
GGTGTGCCAG	CAGTGAATGT	TTGCCCTGAA	TGCACACCAA	GGGCCCCACC	3600

TGCCACAGGA	CACATAGGAC	TCCACAGAGT	CTGGCCTCAC	CTCCCTACTG	3650
TCAGTCTGT	AGAATCGACC	TCTGCTGGCC	GGCTGTACCC	TGAGTACCCT	3700
CTCACTTCT	CCTTCAGGTT	TTCAGGGGAC	AGGCCAAGCC	AGAGGACAGG	3750
ATTCCTTGA	GGCCACAGAG	GAGCACCAG	GAGAAGATCT	GTAAGTAGGC	3800
CTTTGTTAGA	GTCTCCAAGG	TTCAGTTCTC	AGCTGAGGCC	TCTCACACAC	3850
TCCCTCTCTC	CCCAGGCCTG	TGGGTCTTCA	TTGCCACAGT	CCTGCCCAACA	3900
CTCCTGCCTG	CTGCCCTGAC	GAGAGTCATC			3930
ATG TCT CTT	GAG CAG AGG	AGT CTG CAC	TGC AAG CCT	GAG GAA	3972
GCC CTT GAG	GCC CAA CAA	GAG GCC CTG	GGC CTG GTG	TGT GTG	4014
CAG GCT GCC	ACC TCC TCC	TCT TGT CCT	CTG GTC CTG	GGC ACC	4056
CTG GAG GAG	GTG CCC ACT	GCT GGG TCA	ACA GAT CCT	CCC CAG	4098
AGT CCT CAG	GGA GCC TCC	GCC TTT CCC	ACT ACC ATC	AAC TTC	4140
ACT CGA CAG	AGG CAA CCC	AGT GAG GGT	TCC AGC AGC	CGT GAA	4182
GAG GAG GGG	CCA AGC ACC	TCT TGT ATC	CTG GAG TCC	TTG TTC	4224
CGA GCA GTA	ATC ACT AAG	AAG GTG GCT	GAT TTG GTT	GGT TTT	4266
CTG CTC CTC	AAA TAT CGA	GCC AGG GAG	CCA GTC ACA	AAG GCA	4308
GAA ATG CTG	GAG AGT GTC	ATC AAA AAT	TAC AAG CAC	TGT TTT	4350
CCT GAG ATC	TTC GGC AAA	GCC TCT GAG	TCC TTG CAG	CTG GTC	4392
TTT GGC ATT	GAC GTG AAG	GAA GCA GAC	CCC ACC GGC	CAC TCC	4434
TAT GTC CTT	GTC ACC TGC	CTA GGT CTC	TCC TAT GAT	GGC CTG	4476
CTG GGT GAT	AAT CAG ATC	ATG CCC AAG	ACA GGC TTC	CTG ATA	4518
ATT GTC CTG	GTC ATG ATT	GCA ATG GAG	GGC GGC CAT	GCT CCT	4560
GAG GAG GAA	ATC TGG GAG	GAG CTG AGT	GTG ATG GAG	GTG TAT	4602
GAT GGG AGG	GAG CAC AGT	GCC TAT GGG	GAG CCC AGG	AAG CTG	4644
CTC ACC CAA	GAT TTG GTG	CAG GAA AAG	TAC CTG GAG	TAC GGC	4686
AGG TGC CGG	ACA GTG ATC	CCG CAC GCT	ATG AGT TCC	TGT GGG	4728
GTC CAA GGG	CCC TCG CTG	AAA CCA GCT	ATG TGA		4761
AAGTCCTTGA	GTATGTGATC	AAGGTCAGTG	CAAGAGTTC		4800
GCTTTTCTT	CCCATCCCTG	CGTGAAGCAG	CTTTGAGAGA	GGAGGAAGAG	4850
GGAGTCTGAG	CATGAGTTGC	AGCCAAGGCC	AGTGGGAGGG	GGACTGGGCC	4900
AGTGCACCTT	CCAGGGCCGC	GTCCAGCAGC	TTCCCTGCC	TCGTGTGACA	4950
TGAGGCCCCAT	TCTTCACTCT	GAAGAGAGCG	GTCAGTGTTT	TCAGTAGTAG	5000
GTTTCTGTTT	TATTGGGTGA	CTTGGAGATT	TATCTTTGTT	CTCTTTTGGA	5050
ATTGTTCAAA	TGTTTTTTTT	TAAGGGATGG	TTGAATGAAC	TTCAGCATCC	5100
AAGTTTATGA	ATGACAGCAG	TCACACAGTT	CTGTGTATAT	AGTTTAAGGG	5150
TAAGAGTCTT	GTGTTTATT	CAGATTGGGA	AATCCATTCT	ATTTTGTGAA	5200
TTGGGATAAT	AACAGCAGTG	GAATAAGTAC	TTAGAAATGT	GAAAAATGAG	5250
CAGTAAAATA	GATGAGATAA	AGAACTAAAG	AAATTAAGAG	ATAGTCAATT	5300
CTTGCTTTAT	ACCTCAGTCT	ATTCTGTAAA	ATTTTAAAG	ATATATGCAT	5350
ACCTGGATTT	CCTTGCTTTC	TTTGAGAATG	TAAGAGAAAT	TAAATCTGAA	5400
TAAAGAATTC	TTCTGTTCAT	CTGGCTCTTT	TCTTCTCCAT	GCACTGAGCA	5450
TCTGCTTTTT	GGAAGGCCCT	GGGTTAGTAG	TGGAGATGCT	AAGGTAAGCC	5500
AGACTCATAC	CCACCCATAG	GGTCGTAGAG	TCTAGGAGCT	GCAGTCACGT	5550
AATCGAGGTG	GCAAGATGTC	CTCTAAAGAT	GTAGGGAAAA	GTGAGAGAGG	5600
GGTGAGGGTG	TGGGGCTCCG	GGTGAGAGTG	GTGGAGTGTC	AATGCCCTGA	5650
GCTGGGGCAT	TTTGGGCTTT	GGGAACTGC	AGTTCCTTCT	GGGGGAGCTG	5700
ATTGTAATGA	TCTTGGGTGG	ATCC			5724

(2) INFORMATION FOR SEQUENCE ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4157 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-2 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CCCATCCAGA	TCCCCATCCG	GGCAGAATCC	GGTTCCACCC	TTGCCGTGAA	50
CCCAGGGAAG	TCACGGGGCC	GGATGTGACG	CCACTGACTT	GCACATTGGA	100
GGTCAGAGGA	CAGCGAGATT	CTCGCCCTGA	GCAACGGCCT	GACGTCGGCG	150
GAGGGAAGCA	GGCGCAGGCT	CCGTGAGGAG	GCAAGGTAAG	ACGCCGAGGG	200
AGGACTGAGG	CGGGCCTCAC	CCCAGACAGA	GGGCCCCCAA	TTAATCCAGC	250

GCTGCCTCTG	CTGCCGGGCC	TGGACCACCC	TGCAGGGGAA	GACTTCTCAG	300
GCTCAGTCGC	CACCACCTCA	CCCCGCCACC	CCCCGCCGCT	TTAACCCGAG	350
GGAACTCTGG	CGTAAGAGCT	TTGTGTGACC	AGGGCAGGGC	TGGTTAGAAG	400
TGCTCAGGGC	CCAGACTCAG	CCAGGAATCA	AGGTCAGGAC	CCCAAGAGGG	450
GACTGAGGGC	AACCCACCCC	CTACCCCTAC	TACCAATCCC	ATCCCCCAAC	500
ACCAACCCCA	CCCCATCCC	TCAAACACCA	ACCCACCCCC	CAAACCCCAT	550
TCCCATCTCC	TCCCCACCA	CCATCCTGGC	AGAATCCGGC	TTTGCCCTTG	600
CAATCAACCC	ACGGAAGCTC	CGGGAATGGC	GGCCAAGCAC	GCGGATCCTG	650
ACGTTTCACAT	GTACGGCTAA	GGGAGGGAAG	GGGTTGGGTC	TCGTGAGTAT	700
GGCCTTTGGG	ATGCAGAGGA	AGGGCCCAGG	CCTCCTGGAA	GACAGTGGAG	750
TCCTTAGGGG	ACCCAGCATG	CCAGGACAGG	GGGCCCCTG	TACCCCTGTC	800
TCAAACCTGAG	CCACCTTTTC	ATTCAGCCGA	GGGAATCCTA	GGGATGCAGA	850
CCCACTTCAG	GGGTTGGGG	CCCAGCCTGC	GAGGAGTCAA	GGGGAGGAAG	900
AAGAGGGAGG	ACTGAGGGGA	CCTTGGAGTC	CAGATCAGTG	GCAACCTTGG	950
GCTGGGGGAT	CCTGGGCACA	GTGGCCGAAT	GTGCCCCGTG	CTCATTGCAC	1000
CTTCAGGGTG	ACAGAGAGTT	GAGGGCTGTG	GCTGTAGGGC	TGGGACTTCA	1050
GGTCAGCAGA	GGGAGGAATC	CCAGGATCTG	CCGGACCCAA	GGTGTGCCCC	1100
CTTCATCAGG	ACTCCCCATA	CCCCCGGCC	AGAAAGAAGG	GATGCCACAG	1150
AGTCTGGAAG	TAAATTGTTC	TTAGCTCTGG	GGGAACCTGA	TCAGGGATGG	1200
CCCTAAGTGA	CAATCTCATT	TGTACCACAG	GCAGGAGGTT	GGGGAACCCCT	1250
CAGGGAGATA	AGGTGTTGGT	GTAAAGAGGA	GCTGTCTGCT	CATTTGAGGG	1300
GGTTCCCCCT	TGAGAAAGGG	CAGTCCCTTG	CAGGAGTAAA	GATGAGTAAC	1350
CCACAGGAGG	CCATCATAAC	GTTACCCCTA	GAACCAAAGG	GGTCAGCCCT	1400
GGACAACGCA	CGTGGGGTAA	CAGGATGTGG	CCCCTCCTCA	CTGTCTTTTC	1450
CAGATCTCAG	GGAGTTGATG	ACCTTGTTTT	CAGAAGGTGA	CTCAGTCAAC	1500
ACAGGGGCCC	CTCTGGTCGA	CAGATGCAGT	GGTCTAGGA	TCTGCCAAGC	1550
ATCCAGGTGG	AGAGCCTGAG	GTAGGATTGA	GGGTACCCCT	GGGCCAGAAT	1600
GCAGCAAGGG	GGCCCCATAG	AAATCTGCCC	TGCCCCCTGG	GTTACTTCAG	1650
AGACCCCTGG	CAGGGCTGTC	AGCTGAAGTC	CCTCCATTAT	CTGGGATCCT	1700
TGATGTCAGG	GAAGGGGAGG	CCTTGGTCTG	AAGGGGCTGG	AGTCAGGTCA	1750
GTAGAGGGAG	GGTCTCAGGC	CCTGCCAGGA	GTGGACGTGA	GGACCAAGCG	1800
GACTCGTCAC	CCAGGACACC	TGGACTCCAA	TGAATTTGAC	ATCTCTCGTT	1850
GTCTTTCGCG	GAGGACCTGG	TCACGTATGG	CCAGATGTGG	GTCCCCCTCA	1900
TCTCCTTCGT	TACCATATCA	GGGATGTGAG	TTCTTGACAT	GAGAGATTCT	1950
CAAGCCAGCA	AAAGGGTGGG	ATTAGGCCCT	ACAAGGAGAA	AGGTGAGGGC	2000
CCTGAGTGAG	CACAGAGGGG	ACCCTCCACC	CAAGTAGAGT	GGGGACCTCA	2050
CGGAGTCTGG	CCAACCCCTG	TGAGACTTCT	GGGAATCCGT	GGCTGTGCTT	2100
GCAGTCTGCA	CAGTGAAGGC	CCGTGCATTC	CTCTCCCAGG	AATCAGGAGC	2150
TCCAGGAACC	AGGCAGTGAG	GCCTTGGTCT	GAGTCAGTGC	CTCAGGTAC	2200
AGAGCAGAGG	GGACGCAGAC	AGTGCCAACA	CTGAAGGTTT	GCCTGGAATG	2250
CACACCAAGG	GCCCCACCCG	CCCAGAACAA	ATGGGACTCC	AGAGGGCCTG	2300
GCCTCACCCCT	CCCTATTCTC	AGTCTGTCAG	CCTGAGCATG	TGCTGGCCGG	2350
CTGTACCCCTG	AGGTGCCCTC	CCACTTCCTC	CTTCAGGTTT	TGAGGGGGAC	2400
AGGCTGACAA	GTAGGACCCG	AGGCACTGGA	GGAGCATTGA	AGGAGAAGAT	2450
CTGTAAGTAA	GCCTTTGTCA	GAGCCTCCAA	GGTTTCAGTT	AGTTCTCACC	2500
TAAAGCCTCA	CACACGCTCC	TTCTCTCCCC	AGGCCTGTGG	GTCTTCATTG	2550
CCCAGCTCCT	GCCCCGACTC	CTGCCCTGCT	CCCTGACCAG	AGTCATC	2597
ATG CCT CTT	GAG CAG AGG	AGT CAG CAC	TGC AAG CCT	GAA GAA	2639
GGC CTT GAG	GCC CGA GGA	GAG GCC CTG	GGC CTG GTG	GGT GCG	2681
CAG GCT CCT	GCT ACT GAG	GAG CAG CAG	ACC GCT TCT	TCC TCT	2723
TCT ACT CTA	GTG GAA GTT	ACC CTG GGG	GAG GTG CCT	GCT GCC	2765
GAC TCA CCG	AGT CCT CCC	CAC AGT CCT	CAG GGA GCC	TCC AGC	2807
TTC TCG ACT	ACC ATC AAC	TAC ACT CTT	TGG AGA CAA	TCC GAT	2849
GAG GGC TCC	AGC AAC CAA	GAA GAG GAG	GGG CCA AGA	ATG TTT	2891
CCC GAC CTG	GAG TCC GAG	TTC CAA GCA	GCA ATC AGT	AGG AAG	2933
ATG GTT GAG	TTG GTT CAT	TTT CTG CTC	CTC AAG TAT	CGA GCC	2975
AGG GAG CCG	GTC ACA AAG	GCA GAA ATG	CTG GAG AGT	GTC CTC	3017
AGA AAT TGC	CAG GAC TTC	TTT CCC GTG	ATC TTC AGC	AAA GCC	3059
TCC GAG TAC	TTG CAG CTG	GTC TTT GGC	ATC GAG GTG	GTG GAA	3101
GTG GTC CCC	ATC AGC CAC	TTG TAC ATC	CTT GTC ACC	TGC CTG	3143
GGC CTC TCC	TAC GAT GGC	CTG CTG GGC	GAC AAT CAG	GTC ATG	3185
CCC AAG ACA	GGC CTC CTG	ATA ATC GTC	CTG GCC ATA	ATC GCA	3227
ATA GAG GGC	GAC TGT GCC	CCT GAG GAG	AAA ATC TGG	GAG GAG	3269
CTG AGT ATG	TTG GAG GTG	TTT GAG GGG	AGG GAG GAC	AGT GTC	3311
TTC GCA CAT	CCC AGG AAG	CTG CTC ATG	CAA GAT CTG	GTG CAG	3353
GAA AAC TAC	CTG GAG TAC	CGG CAG GTG	CCC GGC AGT	GAT CCT	3395

GCA TGC TAC GAG TTC CTG TGG GGT CCA AGG GCC CTC ATT GAA	3437
ACC AGC TAT GTG AAA GTC CTG CAC CAT ACA CTA AAG ATC GGT	3479
GGA GAA CCT CAC ATT TCC TAC CCA CCC CTG CAT GAA CGG GCT	3521
TTG AGA GAG GGA GAA GAG TGA	3542
GTCTCAGCAC ATGTTGCAGC CAGGGCCAGT GGGAGGGGGT CTGGGCCAGT	3592
GCACCTTCCA GGGCCCCATC CATTAGCTTC CACTGCCTCG TGTGATATGA	3642
GGCCCATTC TGCCTCTTTG AAGAGAGCAG TCAGCATTCT TAGCAGTGAG	3692
TTTCTGTTCT GTTGATGAC TTTGAGATT ATCTTTCTTT CCTGTTGAA	3742
TTGTTCAAAT GTTCCTTTTA ACAAATGGTT GGATGAACCT CAGCATCCAA	3792
GTTTATGAAT GACAGTAGTC ACACATAGTG CTGTTTATAT AGTTTAGGGG	3842
TAAGAGTCCT GTTTTTTATT CAGATTGGGA AATCCATTCC ATTTTGTGAG	3892
TTGTCACATA ATAACAGCAG TGGAAATATG ATTTGCCTAT ATTGTGAACG	3942
AATTAGCAGT AAAATACATG ATACAAGGAA CTCAAAAGAT AGTTAATTCT	3992
TGCCTTATAC CTCAGTCTAT TATGTAAAAT TAAAAATATG TGTATGTTTT	4042
TGCTTCTTTG AGAATGCAAA AGAAATTAAA TCTGAATAAA TTCTTCCTGT	4092
TCCTGGCTC ATTCTTTAC CATTCACTCA GCATCTGCTC TGTGGAAGGC	4142
CCTGGTAGTA GTGGG	4157

(2) INFORMATION FOR SEQUENCE ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 662 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-21 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGATCCCCAT GGATCCAGGA AGAATCCAGT TCCACCCCTG CTGTGAACCC	50
AGGGAAGTCA CGGGGCCGGA TGTGACGCCA CTGACTTGCG CGTTGGAGGT	100
CAGAGAACAG CGAGATTCTC GCCCTGAGCA ACGGCCTGAC GTCGGCGGAG	150
GGAAGCAGGC GCAGGCTCCG TGAGGAGGCA AGGTAAGATG CCGAGGGAGG	200
ACTGAGGCGG GCCTCACCCC AGACAGAGGG CCCCATAATA TCCAGCGCTG	250
CCTCTGCTGC CAGGCCTGGA CCACCCTGCA GGGGAAGACT TCTCAGGCTC	300
AGTCGCCACC ACCTACCCC GCCACCCCC GCGGCTTTAA CCGCAGGGAA	350
CTCTGGTGTA AGAGCTTTGT GTGACCAGGG CAGGGCTGGT TAGAAGTGCT	400
CAGGGCCAG ACTCAGCCAG GAATCAAGGT CAGGACCCCA AGAGGGGACT	450
GAGGGTAACC CCCCAGCAC CCCACCACCA TTCCCATCCC CCAACACCAA	500
CCCCACCCC ATCCCCAAC ACCAAACCCA CCACCATCGC TCAAACATCA	550
ACGGCACCCC CAAACCCCGA TTCCCATCCC CACCCATCCT GGCAGATCG	600
GAGCTTTGCC CCTGCAATCA ACCCACGGAA GCTCCGGGAA TGGCGGCCAA	650
GCACGCGGAT CC	662

(2) INFORMATION FOR SEQUENCE ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1640 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:

(A) NAME/KEY: cDNA MAGE-3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GCCGCGAGGG AAGCCGGCCC AGGCTCGGTG AGGAGGCAAG GTTCTGAGGG	50
GACAGGCTGA CCTGGAGGAC CAGAGGCCCC CGGAGGAGCA CTGAAGGAGA	100
AGATCTGCCA GTGGGTCTCC ATTGCCAGC TCCTGCCAC ACTCCCSCCT	150
GTTGCCCTGA CCAGAGTCAT C	171
ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA	213
GGC CTT GAG GCC CGA GGA GAG GCC CTG GGC CTG GTG GGT GCG	255
CAG GCT CCT GCT ACT GAG GAG CAG GAG GCT GCC TCC TCC TCT	297
TCT ACT CTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC	339

GAG TCA CCA GAT CCT CCC CAG AGT CCT CAG GGA GCC TCC AGC	381
CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT	423
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC	465
CCT GAC CTG GAG TCC GAG TTC CAA GCA GCA CTC AGT AGG AAG	507
GTG GCC GAG TTG GTT CAT TTT CTG CTC CTC AAG TAT CGA GCC	549
AGG GAG CCG GTC ACA AAG GCA GAA ATG CTG GGG AGT GTC GTC	591
GGA AAT TGG CAG TAT TTC TTT CCT GTG ATC TTC AGC AAA GCT	633
TCC AGT TCC TTG CAG CTG GTC TTT GGC ATC GAG CTG ATG GAA	675
GTG GAC CCC ATC GGC CAC TTG TAC ATC TTT GCC ACC TGC CTG	717
GGC CTC TCC TAC GAT GGC CTG CTG GGT GAC AAT CAG ATC ATG	759
CCC AAG GCA GGC CTC CTG ATA ATC GTC CTG GCC ATA ATC GCA	801
AGA GAG GGC GAC TGT GCC CCT GAG GAG AAA ATC TGG GAG GAG	843
CTG AGT GTG TTA GAG GTG TTT GAG GGG AGG GAA GAC AGT ATG	885
TTG GGG GAT CCC AAG AAG CTG CTC ACC CAA CAT TTC GTG CAG	927
GAA AAC TAC CTG GAG TAC CGG CAG GTC CCC GGC AGT GAT CCT	969
GCA TGT TAT GAA TTC CTG TGG GGT CCA AGG GCC CTC GTT GAA	1011
ACC AGC TAT GTG AAA GTC CTG CAC CAT ATG GTA AAG ATC AGT	1053
GGA GGA CCT CAC ATT TCC TAC CCA CCC CTG CAT GAG TGG GTT	1095
TTG AGA GAG GGG GAA GAG TGA	1116
GTCTGAGCAC GAGTTGCAGC CAGGGCCAGT GGGAGGGGGT CTGGGCCAGT	1166
GCACCTTCCG GGGCCGCATC CCTTAGTTTC CACTGCCTCC TGTGACGTGA	1216
GGCCCATCTC TCACTCTTTG AAGCGAGCAG TCAGCATTCT TAGTAGTGGG	1266
TTTCTGTTCT GTTGATGAC TTTGAGATTA TTCTTTGTTT CCTGTTGGAG	1316
TTGTTCAAAAT GTTCCTTTTA ACGGATGGTT GAATGAGCGT CAGCATCCAG	1366
GTTTATGAAT GACAGTAGTC ACACATAGTG CTGTTTATAT AGTTTAGGAG	1416
TAAGAGTCTT GttTTTTACT CAAATTgGGA AATCCATTCC ATTTTGTGAA	1466
TTGTGACATA ATAATAGCAG TGGTAAAAGT ATTTGCTTAA AATTGTGAGC	1516
GATAGTGCAA TAACATACAT GAGATAACTC AAGAAATCAA AAGATAGTTG	1566
ATTCTTGCCCT TGTACCTCAA TCTATTCTGT AAAATTAAAC AAATATGCAA	1616
ACCAGGATT CTTGACTTC TTTG	1640

(2) INFORMATION FOR SEQUENCE ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 943 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-31 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGATCCTCCA CCCAGTAGA GTGGGGACCT CACAGAGTCT GGCCAACCTT	50
CCTGACAGTT CTGGGAATCC GTGGCTGCGT TTGCTGTCTG CACATTGGGG	100
GCCCGTGGAT TCCTCTCCCA GGAATCAGGA GCTCCAGGAA CAAGGCAGTG	150
AGGACTTGGT CTGAGGCAGT GTCCTCAGGT CACAGAGTAG AGGGGgCTCA	200
GATAGTGCCA ACGGTGAAGG TTGCTTGG ATTCAAACCA AGGGCCCCAC	250
CTGCCCCAGA ACACATGGAC TCCAGAGCGC CTGGCCTCAC CCTCAATACT	300
TTCAGTCCTG CAGCCTCAGC ATGCGCTGGC CGGATGTACC CTGAGGTGCC	350
CTCTCACTTC CTCCTTCAGG TTCTGAGGGG ACAGGCTGAC CTGGAGGACC	400
AGAGGCCCCC GGAGGAGCAC TGAAGGAGAA GATCTGTAAG TAAGCCTTTG	450
TTAGAGCCTC CAAGGTTCCA TTCAGTACTC AGCTGAGGTC TCTCACATGC	500
TCCCTCTCTC CCCAGGCCAG TGGGTCTCCA TGCCCCAGCT CCTGCCACCA	550
CTCCCGCCTG TTGCCCTGAC CAGAGTCATC	580
ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA	622
GGC CTT GAG GCC CGA GGA GAg GCC CTG GGC CTG GTG GGT GCG	664
CAG GCT CCT GCT ACT GAG GAG CAG GAG GCT GCC TCC TCC TCT	706
TCT AGT GTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC	748
GAG TCA CCA GAT CCT CCC CAG AGT CCT CAG GGA GCC TCC AGC	790
CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT	832
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC	874
CCT GAC CTG GAG TCT GAG TTC CAA GCA GCA CTC AGT AGG AAG	916
GTG GCC AAG TTG GTT CAT TTT CTG CTC	943

- (2) INFORMATION FOR SEQUENCE ID NO: 13:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2531 base pairs
 (B) TYPE: nucleic acid
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: genomic DNA
 (ix) FEATURE:
 (A) NAME/KEY: MAGE-4 gene
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

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GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG      50
GGGATCATCC ACTCCATGAG AGTGGGGACC TCACAGAGTC CAGCCTACCC      100
TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG      150
GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT      200
TGGTCTGAGA CAGTGTCTCT AGGTTACAGA GCAGAGGATG CACAGGCTGT      250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA      300
CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT      350
CCTGCAGAAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA      400
CTTCCTCCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC      450
TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT      500
TAGAGCCTCT AAGATTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC      550
TCTCCGTAGG CCTGTGGGTC CCCATTGCC AGCTTTTGCC TGCACCTCTG      600
CCTGCTGCCC TGACCAGAGT CATC                                     624
ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA      666
GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCA      708
CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC      750
TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT      792
GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT      834
GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC      876
AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC      918
TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC      960
AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA     1002
GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC     1044
ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA     1086
GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG     1128
GAA GTG GAC CCC GCC AGC AAC ACC TAC ACC CTT GTC ACC TGC     1170
CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC     1212
TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT     1254
GCA ATG GAG GGC GAC AGC GCC TCT GAG GAG GAA ATC TGG GAG     1296
GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT     1338
GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG     1380
CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT     1422
CCT CGC CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT     1464
GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC     1506
AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA     1548
GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA                       1578
GCATGAGTTG CAGCCAGGGC TGTGGGGAAG GGGCAGGGCT GGGCCAGTGC     1628
ATCTAACAGC CCTGTGCAGC AGCTTCCCTT GCCTCGTGTA ACATGAGGCC     1678
CATTCTTCAC TCTGTTTGAA GAAAATAGTC AGTGTTCTTA GTAGTGGGTT     1728
TCTATTTTGT TGGATGACTT GGAGATTAT CTCTGTTTCC TTTTACAATT     1778
GTTGAAATGT TCCTTTTAAT GGATGGTTGA ATTAACCTCA GCATCCAAGT     1828
TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG     1878
AGTCTTGTTT TTTATTCAGA TTGGGAAATC CGTTCTATTT TGTGAATTTG     1928
GGACATAATA ACAGCAGTGG AGTAAGTATT TAGAAGTGTG AATTCACCGT     1978
GAAATAGGTG AGATAAATTA AAAGATACTT AATTCCTGCC TTATGCCTCA     2028
GTCTATTCTG TAAAATTAA AAATATATAT GCATACCTGG ATTCCTTGG     2078
CTTCGTGAAT GTAAGAGAAA TTAAATCTGA ATAAATAATT CTTTCTGTTA     2128
ACTGGCTCAT TTCTTCTCTA TGCACCTGAGC ATCTGCTCTG TGGAAGGCCC     2178
AGGATTAGTA GTGGAGATAC TAGGGTAAGC CAGACACACA CCTACCGATA     2228
GGGTATTAAAG AGTCTAGGAG CGCGGTCATA TAATTAAGGT GACAAGATGT     2278
CCTCTAAGAT GTAGGGGAAA AGTAACGAGT GTGGGTATGG GGCTCCAGGT     2328
GAGAGTGGTC GGGTGTAAAT TCCCTGTGTG GGGCCTTTTG GGCTTTGGGA     2378
AACTGCATTT TCTTCTGAGG GATCTGATTC TAATGAAGCT TGGTGGGTCC     2428
AGGGCCAGAT TCTCAGAGGG AGAGGAAAAA GCCCAGATTG GAAAAGTTGC     2478

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TCTGAGCAGT TCCTTTGTGA CAATGGATGA ACAGAGAGGA GCCTCTACCT 2528
 GGG 2531

(2) INFORMATION FOR SEQUENCE ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2531 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-41 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG 50
 GGGATCATCC ACTCCATGAG AGTGGGGACC TCACAGAGTC CAGCCTACCC 100
 TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG 150
 GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT 200
 TGGTCTGAGA CAGTGTCTCT AGGTTACAGA GCAGAGGATG CACAGGCTGT 250
 GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA 300
 CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT 350
 CCTGCAGAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA 400
 CTTCTCTCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC 450
 TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT 500
 TAGAGCCTCT AAGATTTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC 550
 TCTCCGTAGG CCTGTGGGTC CCCATTGCCC AGCTTTTGCC TGCACCTTTG 600
 CCTGCTGCCC TGAGCAGAGT CATC 624
 ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA 666
 GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCG 708
 CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC 750
 TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT 792
 GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT 834
 GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC 876
 AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC 918
 TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC 960
 AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA 1002
 GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC 1044
 ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA 1086
 GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG 1128
 GAA GTG GAC CCC ACC AGC AAC ACC TAC ACC CTT GTC ACC TGC 1170
 CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC 1212
 TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT 1254
 GCA ATG GAG GGC GAC AGC GCC TCT GAG GAG GAA ATC TGG GAG 1296
 GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT 1338
 GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG 1380
 CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT 1422
 CCT GCG CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT 1464
 GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC 1506
 AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA 1548
 GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA 1578
 GCATGAGTTG CAGCCAGGGC TGTGGGGAAG GGGCAGGGCT GGGCCAGTGC 1628
 ATCTAACAGC CCTGTGCAGC AGCTTCCCTT GCCTCGTGTA ACATGAGGCC 1678
 CATCTTTCAC TCTGTTTGAA GAAAATAGTC AGTGTTCTTA GTAGTGGGTT 1728
 TCTATTTTGT TGGATGACTT GGAGATTAT CTCTGTTTCC TTTTACAATT 1778
 GTTGAATGT TCCTTTTAAT GGATGGTTGA ATTAACCTCA GCATCCAAGT 1828
 TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG 1878
 AGTCTTGTT TTTATTCAGA TTGGGAAATC CGTTCTATTT TGTGAATTTG 1928
 GGACATAATA ACAGCAGTGG AGTAAGTATT TAGAAGTGTG AATTACCGT 1978
 GAAATAGGTG AGATAAATTA AAAGATACTT AATTCGCGCC TTATGCCTCA 2028
 GTCTATTCTG TAAAATTTAA AAATATATAT GCATACCTGG ATTTCTTTGG 2078
 CTTCTGTAAT GTAAGAGAAA TTAAATCTGA ATAAATAATT CTTTCTGTGA 2128
 ACTGGCTCAT TTCTTCTCTA TGCACCTGAGC ATCTGCTCTG TGAAGGCCCC 2178
 AGGATTAGTA GTGGAGATAC TAGGGTAAGC CAGACACACA CCTACCGATA 2228

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GGGTATTAAG	AGTCTAGGAG	CGCGGTCATA	TAATTAAGGT	GACAAGATGT	2278
CCTCTAAGAT	GTAGGGGAAA	AGTAACGAGT	GTGGGTATGG	GGCTCCAGGT	2328
GAGAGTGGTC	GGGTGTAAAT	TCCCTGTGTG	GGGCCTTTTG	GGCTTTGGGA	2378
AACTCCATTT	TCTTCTGAGG	GATCTGATTC	TAATGAAGCT	TGGTGGGTCC	2428
AGGGCCAGAT	TCTCAGAGGG	AGAGGGAAAA	GCCCAGATTG	GAAAAGTTGC	2478
TCTGAGCGGT	TCCTTTGTGA	CAATGGATGA	ACAGAGAGGA	GCCTCTACCT	2528
GGG					2531

- (2) INFORMATION FOR SEQUENCE ID NO: 15:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1068 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA to mRNA
 - (ix) FEATURE:
 - (A) NAME/KEY: cDNA MAGE-4
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

G	GGG	CCA	AGC	ACC	TCG	CCT	GAC	GCA	GAG	TCC	TTG	TTC	CGA	40
GAA	GCA	CTC	AGT	AAC	AAG	GTG	GAT	GAG	TTG	GCT	CAT	TTT	CTG	82
CTC	CGC	AAG	TAT	CGA	GCC	AAG	GAG	CTG	GTC	ACA	AAG	GCA	GAA	124
ATG	CTG	GAG	AGA	GTC	ATC	AAA	AAT	TAC	AAG	CGC	TGC	TTT	CCT	166
GTG	ATC	TTC	GGC	AAA	GCC	TCC	GAG	TCC	CTG	AAG	ATG	ATC	TTT	208
GGC	ATT	GAC	GTG	AAG	GAA	GTG	GAC	CCC	GCC	AGC	AAC	ACC	TAC	250
ACC	CTT	GTC	ACC	TGC	CTG	GGC	CTT	TCC	TAT	GAT	GGC	CTG	CTG	292
GGT	AAT	AAT	CAG	ATC	TTT	CCC	AAG	ACA	GGC	CTT	CTG	ATA	ATC	334
GTC	CTG	GGC	ACA	ATT	GCA	ATG	GAG	GGC	GAC	AGC	GCC	TCT	GAG	376
GAG	GAA	ATC	TGG	GAG	GAG	CTG	GGT	GTG	ATG	GGG	GTG	TAT	GAT	418
GGG	AGG	GAG	CAC	ACT	GTC	TAT	GGG	GAG	CCC	AGG	AAA	CTG	CTC	460
ACC	CAA	GAT	TGG	GTG	CAG	GAA	AAC	TAC	CTG	GAG	TAC	CGG	CAG	502
GTA	CCC	GGC	AGT	AAT	CCT	GCG	CGC	TAT	GAG	TTC	CTG	TGG	GGT	544
CCA	AGG	GCT	CTG	GCT	GAA	ACC	AGC	TAT	GTG	AAA	GTC	CTG	GAG	586
CAT	GTG	GTC	AGG	GTC	AAT	GCA	AGA	GTT	CGC	ATT	GCC	TAC	CCA	628
TCC	CTG	CGT	GAA	GCA	GCT	TTG	TTA	GAG	GAG	GAA	GAG	GGA	GTC	670
TGAGCATGAG	TTGCAGCCAG	GGCTGTGGGG	AAGGGGCAGG	GCTGGGCCAG										720
TGCATCTAAC	AGCCCTGTGC	AGCAGCTTCC	CTTGCCTCGT	GTAACATGAG										770
GCCCCATTCTT	CACTCTGTTT	GAAGAAAATA	GTCAGTGTTT	TTAGTAGTGG										820
GTTTCTATTT	TGTTGGATGA	CTTGGAGATT	TATCTCTGTT	TCCTTTTACA										870
ATTGTTGAAA	TGTTCTTTT	AATGGATGGT	TGAATTAAT	TCAGCATCCA										920
AGTTTATGAA	TCGTAGTTAA	CGTATATTGC	TGTTAATATA	GTTTAGGAGT										970
AAGAGTCTTG	TTTTTTATTC	AGATTGGGAA	ATCCGTTCTA	TTTTGTGAAT										1020
TTGGGACATA	ATAACAGCAG	TGGAGTAAGT	ATTTAGAAGT	GTGAATTC										1068

- (2) INFORMATION FOR SEQUENCE ID NO: 16:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2226 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: genomic DNA
 - (ix) FEATURE:
 - (A) NAME/KEY: MAGE-5 gene
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GGATCCAGGC	CTTGCCAGGA	GAAAGGTGAG	GGCCCTGTGT	GAGCACAGAG	50
GGGACCATTG	ACCCCAAGAG	GGTGGAGACC	TCACAGATTC	CAGCCTACCC	100
TCCTGTTAGC	ACTGGGGGCC	TGAGGCTGTG	CTTGCACTCT	GCACCCTGAG	150
GGCCCATGCA	TTCCTCTTCC	AGGAGCTCCA	GGAACAGAC	ACTGAGGCCT	200
TGGTCTGAGG	CCGTGCCCTC	AGGTCACAGA	GCAGAGGAGA	TGCAGACGTC	250
TAGTGCCAGC	AGTGAACGTT	TGCCTTGAAT	GCACACTAAT	GGCCCCCATC	300

GCCCCAGAAC	ATATGGGACT	CCAGAGCACC	TGGCCTCACC	CTCTCTACTG	350
TCAGTCCTGC	AGAATCAGCC	TCTGCTTGCT	TGTGTACCCT	GAGGTGCCCT	400
CTCACTTTTT	CCTTCAGGTT	CTCAGGGGAC	AGGCTGACCA	GGATCACCAG	450
GAAGCTCCAG	AGGATCCCCA	GGAGGCCCTA	GAGGAGCACC	AAAGGAGAAG	500
ATCTGTAAGT	AAGCCTTTGT	TAGAGCCTCC	AAGGTTCACT	TTTGTAGCTGA	550
GGCTTCTCAC	ATGCTCCCTC	TCTCTCCAGG	CCAGTGGGTC	TCCATTGCCC	600
AGCTCCTGCC	CACACTCCTG	CCTGTTGCCG	TGACCAGAGT	CGTC	644
ATG TCT CTT	GAG CAG AAG	AGT CAG CAC	TGC AAG CCT	GAG GAA	684
CTC CTC TGG	TCC CAG GCA	CCC TGG GGG	AGG TGC CTG	CTG CTG	728
GGT CAC CAG	GTG CTC TCA	AGA GTC CTC	AGG GAG CCT	CCG CCA	770
TCC CCA CTG	CCA TCG ATT	TCA CTC TAT	GGA GGC AAT	CCA TTA	812
AGG GCT CCA	GCA ACC AAG	AAG AGG AGG	GGC CAA GCA	CCT CCC	854
CTG ACC CAG	AGT CTG TGT	TCC GAG CAG	CAC TCA GTA	AGA AGG	896
TGG CTG ACT	TGA				908
TTCATTTTCT	GCTCCTCAAG	TATTAAGTCA	AGGAGCTGGT	CACAAAGGCA	958
GAAATGCTGG	AGAGCGTCAT	CAAAAATTAC	AAGCGCTGCT	TTCCTGAGAT	1008
CTTCGGCAAA	GCCTCCGAGT	CCTTGCCAGCT	GGTCTTTGGC	ATTGACGTGA	1058
AGGAAGCGGA	CCCCACCAGC	AACACCTACA	CCCTTGTCAC	CTGCCTGGGA	1108
CTCCTATGAT	GGCCTGCTGG	TTGATAATAA	TCAGATCATG	CCCAAGACGG	1158
GCCTCCTGAT	AATCGTCTTG	GGCATGATTG	CAATGGAGGG	CAAATGCGTC	1208
CCTGAGGAGA	AAATCTGGGA	GGAGCTGAGT	GTGATGAAGG	TGTATGTTGG	1258
GAGGGAGCAC	AGTGTCTGTG	GGGAGCCCAG	GAAGCTGCTC	ACCCAAGATT	1308
TGGTGACGGA	AAACTACCTG	GAGTACCGGC	AGGTGCCAG	CAGTGATCCC	1358
ATATGCTATG	AGTTACTGTG	GGGTCCAAGG	GCACTCGCTG	CTTGAAAGTA	1408
CTGGAGCACG	TGGTCAGGGT	CAATGCAAGA	GTTCTCATT	CCTACCCATC	1458
CCTGCGTGAA	GCAGCTTTGA	GAGAGGAGGA	AGAGGGAGTC	TGAGCATGAG	1508
CTGCAGCCAG	GGCCACTGCC	AGGGGGGCTG	GGCCAGTGCA	CCTTCCAGGG	1558
CTCCGCTCAG	TAGTTTCCCC	TGCCTTAATG	TGACATGAGG	CCCATTCTTC	1608
TCTCTTTGAA	GAGAGCAGTC	AACATTCTTA	GTAGTGGGTT	TCTGTTCTAT	1658
TGGATGACTT	TGAGATTGT	CTTTGTTTCC	TTTTGGAATT	GTTCAAATGT	1708
TTCTTTTAAT	GGGTGGTTGA	ATGAACCTCA	GCATTCAAAT	TTATGAATGA	1758
CAGTAGTCAC	ACATAGTGCT	GTTTATATAG	TTTAGGAGTA	AGAGTCTTGT	1808
TTTTTTATTCA	GATTGGGAAA	TCCATTCCAT	TTTGTGAATT	GGGACATAGT	1858
TACAGCAGTG	GAATAAGTAT	TCATTTAGAA	ATGTGAATGA	GCAGTAAAAC	1908
TGATGACATA	AAGAAATTAA	AAGATATTTA	ATTCTTGCTT	ATACTCAGTC	1958
TATTCGGTAA	AATTTTTTTT	AAAAAATGTG	CATACCTGGA	TTTCCTTGGC	2008
TTCTTTGAGA	ATGTAAGACA	AATTAATCT	GAATAAATCA	TTCTCCCTGT	2058
TCACTGGCTC	ATTTATTCTC	TATGCACTGA	GCATTTGCTC	TGTGGAAGGC	2108
CCTGGGTTAA	TAGTGGAGAT	GCTAAGGTAA	GCCAGACTCA	CCCCTACCCA	2158
CAGGGTAGTA	AAGTCTAGGA	GCAGCAGTCA	TATAATTAAG	GTGGAGAGAT	2208
GCCCTCTAAG	ATGTAGAG				2226

(2) INFORMATION FOR SEQUENCE ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2305 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-51 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGATCCAGGC	CTTGCCAGGA	GAAAGGTGAG	GGCCCTGTGT	GAGCACAGAG	50
GGGACCATT	ACCCCAAGAG	GGTGGAGACC	TCACAGATT	CAGCCTACCC	100
TCCTGTTAG	ACTGGGGGCC	TGAGGCTGTG	CTTGCACTCT	GCACCCTGAG	150
GGCCCATGCA	TTCCTCTTCC	AGGAGCTCCA	GGAAACAGAC	ACTGAGGCCT	200
TGGTCTGAG	CCGTGCCCTC	AGGTCACAGA	GCAGAGGAGA	TGCAGACGTC	250
TAGTGCACG	AGTGAACGTT	TGCCTTGAAT	GCACACTAAT	GGCCCCATC	300
GCCCCAGAAC	ATATGGGACT	CCAGAGCACC	TGGCCTCACC	CTCTCTACTG	350
TCAGTCCTGC	AGAATCAGCC	TCTGCTTGCT	TGTGTACCCT	GAGGTGCCCT	400
CTCACTTTTT	CCTTCAGGTT	CTCAGGGGAC	AGGCTGACCA	GGATCACCAG	450
GAAGCTCCAG	AGGATCCCCA	GGAGGCCCTA	GAGGAGCACC	AAAGGAGAAG	500
ATCTGTAAGT	AAGCCTTTGT	TAGAGCCTCC	AAGGTTCACT	TTTGTAGCTGA	550

GGCTTCTCAC	ATGCTCCCTC	TCTCTCCAGG	CCAGTGGGTC	TCCATTGCCC	600
AGCTCCTGCC	CACACTCCTG	CCTGTTGCGG	TGACCAGAGT	CGTC	644
ATG TCT CTT	GAG CAG AAG	AGT CAG CAC	TGC AAG	CCT GAG GAA	686
GGC CTT GAC	ACC CAA GAA	GAG CCC	TGG GCC	TGG TGG GTG TGC	728
AGG CTG CCA	CTA CTG AGG	AGC AGG	CTG TGT	CCT CCT CCT	770
CTC CTC TGG	TCC CAG GCA	CCC TGG	GGG AGG	TGC CTG CTG CTG	812
GGT CAC CAG	GTC CTC TCA	AGA GTC	CTC AGG	GAG CCT CCG CCA	854
TCC CCA CTG	CCA TCG ATT	TCA CTC	TAT GGA	GGC AAT CCA TTA	896
AGG GCT CCA	GCA ACC AAG	AAG AGG	AGG GGC	CAA GCA CCT CCC	938
CTG ACC CAG	AGT CTG TGT	TCC GAG	CAG CAC	TCA GTA AGA AGG	980
TGG CTG ACT	TGA				992
TTCATTTTCT	GCTCCTCAAG	TATTAAGTCA	AGGAGCCGGT	CACAAAGGCA	1042
GAAATGCTGG	AGAGCGTCAT	CAAAAATTAC	AAGCGCTGCT	TTCCTGAGAT	1092
CTTCGGCAAA	GCCTCCGAGT	CCTTGCAGCT	GGTCTTTGGC	ATTGACGTGA	1142
AGGAAGCGGA	CCCCACCAGC	AACACCTACA	CCCTTGTCAC	CTGCCCTGGGA	1192
CTCCTATGAT	GGCCTGGTGG	TTTAATCAGA	TCATGCCCAA	GACGGGCCTC	1242
CTGATAATCG	TCTTGGGCAT	GATTGCAATG	GAGGGCAAAT	GCGTCCCTGA	1292
GGAGAAAATC	TGGGAGGAGC	TGGGTGTGAT	GAAGGTGTAT	GTTGGGAGGG	1342
AGCACAGTGT	CTGTGGGGAG	CCCAGGAAGC	TGCTCACCCA	AGATTTGGTG	1392
CAGGAAAAC	ACCTGGAGTA	CCGCAGGTGC	CCAGCAGTGA	TCCCATATGC	1442
TATGAGTTAC	TGTGGGGTCC	AAGGGCACTC	GCTGCTTGAA	AGTACTGGAG	1492
CACGTGGTCA	GGGTCAATGC	AAGAGTTCTC	ATTTCCTACC	CATCCCTGCA	1542
TGAAGCAGCT	TTGAGAGAGG	AGGAAGAGGG	AGTCTGAGCA	TGAGCTGCAG	1592
CCAGGGCCAC	TGCGAGGGGG	GCTGGGCCAG	TGCACCTTCC	AGGGCTCCGT	1642
CCAGTAGTTT	CCCCTGCCTT	AATGTGACAT	GAGGCCCAT	CTTCTCTCTT	1692
TGAAGAGAGC	AGTCAACATT	CTTAGTAGTG	GGTTTCTGTT	CTATTGGATG	1742
ACTTTGAGAT	TTGTCTTTGT	TTCCTTTTGG	AATTGTTCAA	ATGTTCCCTT	1792
TAATGGGTGG	TTGAATGAAC	TTCAGCATTC	AAATTTATGA	ATGACAGTAG	1842
TCACACATAG	TGCTGTTTAT	ATAGTTTAGG	AGTAAGAGTC	TTGTTTTTTA	1892
TTGAGATTGG	GAAATCCATT	CCATTTTGTG	AATTGGGACA	TAGTTACAGC	1942
AGTGGAATAA	GTATTCATTT	AGAAATGTGA	ATGAGCAGTA	AAACTGATGA	1992
GATAAAGAAA	TTAAAAGATA	TTTAATCTT	GCCTTATACT	CAGTCTATTC	2042
GGTAAATTTT	TTTTTTAAAA	ATGTGCATAC	CTGGATTTC	TTGGCTTCTT	2092
TGAGAATGTA	AGACAAATTA	AATCTGAATA	AATCATTCTC	CCTGTTCACT	2142
GGCTCATTTA	TTCTCTATGC	ACTGAGCATT	TGCTCTGTGG	AAGGCCCTGG	2192
GTTAATAGTG	GAGATGCTAA	GGTAAGCCAG	ACTCACCCCT	ACCCACAGGG	2242
TAGTAAAGTC	TAGGAGCAGC	AGTCATATAA	TTAAAGTGGA	GAGATGCCCT	2292
CTAAGATGTA	GAG				2305

(2) INFORMATION FOR SEQUENCE ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 225 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: MAGE-6 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TAT TTC TTT	CCT GTG ATC	TTC AGC AAA	GCT TCC	GAT TCC TTG	42
CAG CTG GTC	TTT GGC ATC	GAG CTG ATG	GAA GTG	GAC CCC ATC	84
GGC CAC GTG	TAC ATC TTT	GCC ACC TGC	CTG GGC	CTC TCC TAC	126
GAT GGC CTG	CTG GGT GAC	AAT CAG ATC	ATG CCC	AGG ACA GGC	168
TTC CTG ATA	ATC ATC CTG	GCC ATA ATC	GCA AGA	GAG GGC GAC	210
TGT GCC CCT	GAG GAG				225

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- (2) INFORMATION FOR SEQUENCE ID NO: 19:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1947 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: genomic DNA
 - (ix) FEATURE:
 - (A) NAME/KEY: MAGE-7 gene
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TGAATGGACA	ACAAGGGCCC	CACACTCCCC	AGAACAACAG	GGACTCCAGA	50
GAGCCCAGCC	TCACCTTCCC	TACTGTCAGT	CCTGCAGCCT	CAGCCTCTGC	100
TGGCCGGGTG	TACCCTGAGG	TGCCCTCTCA	CTTCCTCCTT	CAGGTTCTCA	150
CGGGACAGGC	CGGCCAGGAG	GTGAGAAGCC	CCAGGAGGCC	CCAGAGGAGC	200
ACCGAAGGAG	AAGATCTGTA	AGTAGGCCCTT	TGTTAGGGCC	TCCAGGGCGT	250
GGTTCACAAA	TGAGGCCCT	CACAAGCTCC	TTCTCTCCCC	AGATCTGTGG	300
GTTCTCTCCC	ATCGCCCAGC	TGCTGCCCGC	ACTCCAGCCT	GCTGCCCTGA	350
CCAGAGTCAT	CATGTCTTCT	GAGCAGAGGA	GTGAGCACTG	CAAGCCTGAG	400
GATGCCCTGA	GGCCCAAGGA	CAGGAGGCTC	TGGGCCTGGT	GGGTGCGCAG	450
GCTCCCGCCA	CCGAGGAGCA	CGAGGCTGCC	TCCTCCTTCA	CTCTGATTGA	500
AGGCACCCTG	GAGGAGGTGC	CTGCTGCTGG	GTCCCCCAGT	CCTCCCCTGA	550
GTCTCAGGCT	TCCTCCTTTT	CCCTGACCAT	CAGCAACAAC	ACTCTATGGA	600
GCCAATCCAG	TGAGGGCACC	AGCAGCCGGG	AAGAGGAGGG	GCCAACCACC	650
TAGACACACC	CCGCTCACCT	GGCGTCCTTG	TTCCA		685
ATG GGA AGG	TGG CTG AGT	TGG TTC GCT	TCC TGC TGC	ACA AGT	727
ATC GAG TCA	AGG AGC TGG	TCA CAA AGG	CAG AAA TGC	TGG ACA	769
GTG TCA TCA	AAA ATT ACA	AGC ACT AGT	TTC CTT GTG	ATC TAT	811
GGC AAA GCC	TCA GAG TGC	ATG CAG GTG	ATG TTT GGC	ATT GAC	853
ATG AAG GAA	GTG GAC CCC	GCG GCC ACT	CCT ACG TCC	TTG TCA	895
CCT GCT TGG	GCC TCT CCT	ACA ATG GCC	TGC TGG GTG	ATG ATC	937
AGA GCA TGC	CCG AGA CCG	GCC TTC TGA			964
TTATGGTCTT	GACCATGATC	TTAATGGAGG	GCCACTGTGC	CCCTGAGGAG	1014
GCAATCTGGG	AAGCGTTGAG	TGTAATGGTG	TATGATGGGA	TGGAGCAGTT	1064
TCTTTGGGCA	GCTGAGGAAG	CTGCTCACCC	AAGATTGGGT	GCAGGAAAAC	1114
TACCTGCAAT	ACCGCCAGGT	GCCCAGCAGT	GATCCCCCGT	GCTACCAATT	1164
CCTGTGGGGT	CCAAGGGCCC	TCATTGAAAC	CAGCTATGTG	AAAGTCCTGG	1214
AGTATGCAGC	CAGGGTCAGT	ACTAAAGAGA	GCATTTCTTA	CCCATCCCTG	1264
CATGAAGAGG	CTTTGGGAGA	GGAGGAAGAG	GGAGTCTGAG	CAGAAGTTGC	1314
AGCCAGGGCC	AGTGGGGCAG	ATTGGGGGAG	GGCCTGGGCA	GTGCACGTTT	1364
CACACATCCA	CCACCTTCCC	TGTCCTGTTA	CATGAGGCCC	ATTCTTCACT	1414
CTGTGTTTGA	AGAGAGCAGT	CAATGTTCTC	AGTAGCGGGG	AGTGTGTTGG	1464
GTGTGAGGGA	ATACAAGGTG	GACCATCTCT	CAGTTCCTGT	TCTCTTGGGC	1514
GATTTGGAGG	TTTATCTTTG	TTTCCTTTTG	CAGTCGTTCA	AATGTTCTTT	1564
TTAATGGATG	GTGTAATGAA	CTTCAACATT	CATTTTATGT	ATGACAGTAG	1614
GCAGACTTAC	TGTTTTTTAT	ATAGTTAAAA	GTAAGTGCAT	TGTTTTTTAT	1664
TTATGTAAGA	AAATCTATGT	TATTTCTTGA	ATTGGGACAA	CATAACATAG	1714
CAGAGGATTA	AGTACCTTTT	ATAATGTGAA	AGAACAAAGC	GGTAAAATGG	1764
GTGAGATAAA	GAAATAAAGA	AATTAAATTG	GCTGGGCACG	GTGGCTCACG	1814
CCTGTAATCC	CAGCACTTTA	GGAGGCAGAG	GCACGGGGAT	CACGAGGTCA	1864
GGAGATCGAG	ACCATTCTGG	CTAACACAGT	GAAACACCAT	CTCTATTAAA	1914
AATACAAAAC	TTAGCCGGGC	GTGGTGGCGG	GTG		1947

- (2) INFORMATION FOR SEQUENCE ID NO: 20:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1810 base pairs
 - (B) TYPE: nucleic acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: genomic DNA
 - (ix) FEATURE:
 - (A) NAME/KEY: MAGE-8 gene
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GAGCTCCAGG	AACCAGGCTG	TGAGGTCTTG	GTCTGAGGCA	GTATCTTCAA	50
TCACAGAGCA	TAAGAGGCCC	AGGCAGTAGT	AGCAGTCAAG	CTGAGGTGGT	100

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GTTTCCCCTG TATGTATACC AGAGGCCCTT CTGGCATCAG AACAGCAGGA 150
ACCCACACAGT TCCTGGCCCT ACCAGCCCTT TTGTCAGTCC TGGAGCCTTG 200
GCCTTTGCCA GGAGGCTGCA CCCTGAGATG CCCTCTCAAT TTCTCCTTCA 250
GGTTCGCAGA GAACAGGCCA GCCAGGAGGT CAGGAGGCCC CAGAGAAGCA 300
CTGAAGAAGA CCTGTAAGTA GACCTTTGTT AGGGCATCCA GGGTGTAGTA 350
CCCAGCTGAG GCCTCTCACA CGCTTCTCT CTCCCAGGC CTGTGGGTCT 400
CAATTGCCCA GCTCCGGCCC AACTCTCTCT GCTGCCCTGA CCTGAGTCAT 450
C 451
ATG CTT CTT GGG CAG AAG AGT CAG CGC TAC AAG GCT GAG GAA 493
GGC CTT CAG GCC CAA GGA GAG GCA CCA GGG CTT ATG GAT GTG 535
CAG ATT CCC ACA GCT GAG GAG CAG AAG GCT GCA TCC TCC TCC 577
TCT ACT CTG ATC ATG GGA ACC CTT GAG GAG GTG ACT GAT TCT 619
GGG TCA CCA AGT CCT CCC CAG AGT CCT GAG GGT GCC TCC TCT 661
TCC CTG ACT GTC ACC GAC AGC ACT CTG TGG AGC CAA TCC GAT 703
GAG GGT TCC AGC AGC AAT GAA GAG GAG GGG CCA AGC ACC TCC 745
CCG GAC CCA GCT CAC CTG GAG TCC CTG TTC CGG GAA GCA CTT 787
GAT GAG AAA GTG GCT GAG TTA GTT CGT TTC CTG CTC CGC AAA 829
TAT CAA ATT AAG GAG CCG GTC ACA AAG GCA GAA ATG CTT GAG 871
AGT GTC ATC AAA AAT TAC AAG AAC CAC TTT CCT GAT ATC TTC 913
AGC AAA GCC TCT GAG TGC ATG CAG GTG ATC TTT GGC ATT GAT 955
GTG AAG GAA GTG GAC CCT GCC GGC CAC TCC TAC ATC CTT GTC 997
ACC TGC CTG GGC CTC TCC TAT GAT GGC CTG CTG GGT GAT GAT 1039
CAG AGT ACG CCC AAG ACC GGC CTC CTG ATA ATC GTC CTG GGC 1081
ATG ATC TTA ATG GAG GGC AGC CGC GCC CCG GAG GAG GCA ATC 1123
TGG GAA GCA TTG AGT GTG ATG GGG GCT GTA TGA 1156
TGGGAGGGAG CACAGTGTCT ATTGGAAGCT CAGGAAGCTG CTCACCCAAG 1206
AGTGGGTGCA GGAGAACTAC CTGGAGTACC GCCAGGCGCC CGGCAGTGAT 1256
CCTGTGCGCT ACGAGTTCCT GTGGGGTCCA AGGGCCCTTG CTGAAACCAG 1306
CTATGTGAAA GTCCTGGAGC ATGTGGTCAG GGTCAATGCA AGAGTTTCGA 1356
TTTCCTACCC ATCCCTGCAAT GAAGAGGCTT TGGGAGAGGA GAAAGGAGTT 1406
TGAGCAGGAG TTGCAGCTAG GGCCAGTGGG GCAGGTGTGT GGAGGGCCTG 1456
GGCCAGTGCA CGTTCCAGGG CCACATCCAC CACTTCCCT GCTCTGTTAC 1506
ATGAGGCCCA TTCTTCACTC TGTGTTTGAA GAGAGCAGTC ACAGTTCTCA 1556
GTAGTGGGGA GCATGTTGGG TGTGAGGGAA CACAGTGTGG ACCATCTCTC 1606
AGTTCTCTGT CTATTGGGCG ATTTGGAGGT TTATCTTTGT TTCCTTTTGG 1656
AATTGTTCCA ATGTTCTTTC TAATGGATGG TGTAATGAAC TTCAACATTC 1706
ATTTTATGTA TGACAGTAGA CAGACTTACT GCTTTTATA TAGTTTAGGA 1756
GTAAGAGTCT TGCTTTTCAT TTATACTGGG AAACCCATGT TATTTCTTGA 1806
ATTC 1810

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(2) INFORMATION FOR SEQUENCE ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1412 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-9 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

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TCTGAGACAG TGTCCTCAGG TCGCAGAGCA GAGGAGACCC AGGCAGTGTC 50
AGCAGTGAAG GTGAAGTGTT CACCCTGAAT GTGCACCAAG GGCCCCACCT 100
GCCCCAGCAC ACATGGGACC CCATAGCACC TGGCCCCATT CCCCCACTG 150
TCACTCATAG AGCCTTGATC TCTGCAGGCT AGCTGCACGC TGAGTAGCCC 200
TCTCACTTCC TCCCTCAGGT TCTCGGGACA GGCTAACCAG GAGGACAGGA 250
GCCCCAAGAG GCCCCAGAGC AGCACTGACG AAGACCTGTA AGTCAGCCTT 300
TGTTAGAACC TCCAAGGTTT GGTCTCAGC TGAAGTCTCT CACACACTCC 350
CTCTCTCCCC AGGCCTGTGG GTCTCCATCG CCCAGTCTCT GCCCACGCTC 400
CTGACTGCTG CCCTGACCAG AGTCATC 427
ATG TCT CTC GAG CAG AGG AGT CCG CAC TGC AAG CCT GAT GAA 469
GAC CTT GAA GCC CAA GGA GAG GAC TTG GGC CTG ATG GGT GCA 511
CAG GAA CCC ACA GGC GAG GAG GAG GAG ACT ACC TCC TCC TCT 553
GAC AGC AAG GAG GAG GAG GTG TCT GCT GCT GGG TCA TCA AGT 595

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CCT CCC CAG AGT CCT CAG GGA GGC GCT TCC TCC TCC ATT TCC	637
GTC TAC TAC ACT TTA TGG AGC CAA TTC GAT GAG GGC TCC AGC	679
AGT CAA GAA GAG GAA GAG CCA AGC TCC TCG GTC GAC CCA GCT	721
CAG CTG GAG TTC ATG TTC CAA GAA GCA CTG AAA TTG AAG GTG	763
GCT GAG TTG GTT CAT TTC CTG CTC CAC AAA TAT CGA GTC AAG	805
GAG CCG GTC ACA AAG GCA GAA ATG CTG GAG AGC GTC ATC AAA	847
AAT TAC AAG CGC TAC TTT CCT GTG ATC TTC GGC AAA GCC TCC	889
GAG TTC ATG CAG GTG ATC TTT GGC ACT GAT GTG AAG GAG GTG	931
GAC CCC GCC GGC CAC TCC TAC ATC CTT GTC ACT GCT CTT GGC	973
CTC TCG TGC GAT AGC ATG CTG GGT GAT GGT CAT AGC ATG CCC	1015
AAG GCC GCC CTC CTG ATC ATT GTC CTG GGT GTG ATC CTA ACC	1057
AAA GAC AAC TGC GCC CCT GAA GAG GTT ATC TGG GAA GCG TTG	1099
AGT GTG ATG GGG GTG TAT GTT GGG AAG GAG CAC ATG TTC TAC	1141
GGG GAG CCC AGG AAG CTG CTC ACC CAA GAT TGG GTG CAG GAA	1183
AAC TAC CTG GAG TAC CCG CAG GTG CCC GGC AGT GAT CCT GCG	1225
CAC TAC GAG TTC CTG TGG GGT TCC AAG GCC CAC GCT GAA ACC	1267
AGC TAT GAG AAG GTC ATA AAT TAT TTG GTC ATG CTC AAT GCA	1309
AGA GAG CCC ATC TGC TAC CCA TCC CTT TAT GAA GAG GTT TTG	1351
GGA GAG GAG CAA GAG GGA GTC TGA	1375
GCACCAGCCG CAGCCGGGGC CAAAGTTTGT GGGGTCA	1412

(2) INFORMATION FOR SEQUENCE ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 920 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-10 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

ACCTGCTCCA GGACAAAGTG GACCCCACTG CATCAGCTCC ACCTACCCTA	50
CTGTCACTCC TGGAGCCTTG GCCTCTGCCG GCTGCATCCT GAGGAGCCAT	100
CTCTCACTTC CTTCTTCAGG TTCTCAGGGG ACAGGGAGAG CAAGAGGTCA	150
AGAGCTGTGG GACACCACAG AGCAGCACTG AAGGAGAAGA CCTGTAAGTT	200
GGCCTTTGTT AGAACCTCCA GGGTGTGGTT CTCAGCTGTG GCCACTTACA	250
CCCTCCCTCT CTCCCCAGGC CTGTGGGTCC CCATCGCCCA AGTCCTGCCC	300
ACACTCCCAC CTGCTACCCCT GATCAGAGTC ATC	333
ATG CCT CGA GCT CCA AAG CGT CAG CGC TGC ATG CCT GAA GAA	375
GAT CTT CAA TCC CAA AGT GAG ACA CAG GGC CTC GAG GGT GCA	417
CAG GCT CCC CTG GCT GTG GAG GAG GAT GCT TCA TCA TCC ACT	459
TCC ACC AGC TCC TCT TTT CCA TCC TCT TTT CCC TCC TCC TCC	501
TCT TCC TCC TCC TCC TCC TGC TAT CCT CTA ATA CCA AGC ACC	543
CCA GAG GAG GTT TCT GCT GAT GAT GAG ACA CCA AAT CCT CCC	585
CAG AGT GCT CAG ATA GCC TGC TCC TCC CCC TCG GTC GTT GCT	627
TCC CTT CCA TTA GAT CAA TCT GAT GAG GGC TCC AGC AGC CAA	669
AAG GAG GAG AGT CCA AGC ACC CTA CAG GTC CTG CCA GAC AGT	711
GAG TCT TTA CCC AGA AGT GAG ATA GAT GAA AAG GTG ACT GAT	753
TTG GTG CAG TTT CTG CTC TTC AAG TAT CAA ATG AAG GAG CCG	795
ATC ACA AAG GCA GAA ATA CTG GAG AGT GTC ATA AAA AAT TAT	837
GAA GAC CAC TTC CCT TTG TTG TTT AGT GAA GCC TCC GAG TGC	879
ATG CTG CTG GTC TTT GGC ATT GAT GTA AAG GAA GTG GAT CC	920

- (2) INFORMATION FOR SEQUENCE ID NO: 23:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1107 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (ix) FEATURE:
- (A) NAME/KEY: MAGE-11 gene
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

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AGAGAACAGG CCAACCTGGA GGACAGGAGT CCCAGGAGAA CCCAGAGGAT      50
CACTGGAGGA GAACAAGTGT AAGTAGGCCT TTGTTAGATT CTCCATGGTT      100
CATATCTCAT CTGAGTCTGT TCTCACGCTC CCTCTCTCCC CAGGCTGTGG      150
GGCCCCATCA CCCAGATATT TCCCACAGTT CGGCCTGCTG ACCTAACCAG      200
AGTCATCATG CCTCTTGAGC AAAGAAGTCA GCACTGCAAG CCTGAGGAAG      250
CCTTCAGGCC CAAGAAGAAG ACCTGGGCCT GGTGGGTGCA CAGGCTCTCC      300
AAGCTGAGGA GCAGGAGGCT GCCTTCTTCT CCTCTACTCT GAATGTGGGC      350
ACTCTAGAGG AGTTGCCTGC TGCTGAGTCA CCAAGTCCTC CCCAGAGTCC      400
TCAGGAAGAG TCCTTCTCTC CCACTGCCAT GGATGCCATC TTTGGGAGCC      450
TATCTGATGA GGGCTCTGGC AGCCAAGAAA AGGAGGGGCC AAGTACCTCG      500
CCTGACCTGA TAGACCCTGA GTCCTTTTCC CAAGATATAC TACATGACAA      550
GATAATTGAT TTGGTTCATT TATTCTCCGC AAGTATCGAG TCAAGGGGCT      600
GATCACAAAG GCAGAA
ATG CTG GGG AGT GTC ATC AAA AAT TAT GAG GAC TAC TTT CCT      658
GAG ATA TTT AGG GAA GCC TCT GTA TGC ATG CAA CTG CTC TTT      700
GGC ATT GAT GTG AAG GAA GTG GAC CCC ACT AGC CAC TCC TAT      742
GTC CTT GTC ACC TCC CTC AAC CTC TCT TAT GAT GGC ATA CAG      784
TGT AAT GAG CAG AGC ATG CCC AAG TCT GGC CTC CTG ATA ATA      826
GTC CTG GGT GTA ATC TTC ATG GAG GGG AAC TGC ATC CCT GAA      868
GAG GTT ATG TGG GAA GTC CTG AGC ATT ATG GGG GTG TAT GCT      910
GGA AGG GAG CAC TTC CTC TTT GGG GAG CCC AAG AGG CTC CTT      952
ACC CAA AAT TGG GTG CAG GAA AAG TAC CTG GTG TAC CGG CAG      994
GTG CCC GGC ACT GAT CCT GCA TGC TAT GAG TTC CTG TGG GGT     1036
CCA AGG GCC CAC GCT GAG ACC AGC AAG ATG AAA GTT CTT GAG     1078
TAC ATA GCC AAT GCC AAT GGG AGG GAT CC                        1107

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- (2) INFORMATION FOR SEQUENCE ID NO: 24:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2150 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (ix) FEATURE:
- (A) NAME/KEY: smage-I
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

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TCTGTCTGCA TATGCCTCCA CTTGTGTGTA GCAGTCTCAA ATGGATCTCT      50
CTCTACAGAC CTCTGTCTGT GTCTGGCACC CTAAGTGGCT TTGCATGGGC      100
ACAGGTTTCT GCCCCTGCAT GGAGCTTAAA TAGATCTTTC TCCACAGGCC      150
TATACCCCTG CATTGTAAGT TTAAGTGGCT TTATGTGGAT ACAGGTCTCT      200
GCCCTTGAT GCAGGCCTAA GTTTTCTGT CTGCTTAACC CCTCCAAGTG      250
AAGCTAGTGA AAGATCTAAC CCACTTTTGG AAGTCTGAAA CTAGACTTTT      300
ATGCAGTGGC CTAACAAGTT TTAATTCTT CCACAGGGTT TGCAGAAAAG      350
AGCTTGATCC ACGAGTTCAG AAGTCCTGTT ATGTTCTTAG AAAG          394
ATG TTC TCC TGG AAA GCT TCA AAA GCC AGG TCT CCA TTA AGT      436
CCA AGG TAT TCT CTA CCT GGT AGT ACA GAG GTA CTT ACA GGT      478
TGT CAT TCT TAT CCT TCC AGA TTC CTG TCT GCC AGC TCT TTT      520
ACT TCA GCC CTG AGC ACA GTC AAC ATG CCT AGG GGT CAA AAG      565
AGT AAG ACC CGC TCC CGT GCA AAA CGA CAG CAG TCA CGC AGG      604
GAG GTT CCA GTA GTT CAG CCC ACT GCA GAG GAA GCA GGG TCT      646
TCT CCT GTT GAC CAG AGT GCT GGG TCC AGC TTC CCT GGT GGT      688

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TCT GCT CCT CAG GGT GTG AAA ACC CCT GGA TCT TTT GGT GCA	730
GGT GTA TCC TGC ACA GGC TCT GGT GGT AGA AAT GCT	772
GCT GTC CTG CCT GAT ACA AAA AGT TCA GAT GGC ACC CAG GCA	814
GGG ACT TCC ATT CAG CAC ACA CTG AAA GAT CCT ATC ATG AGG	856
AAG GCT AGT GTG CTG ATA GAA TTC CTG CTA GAT AAA TTT AAG	898
ATG AAA GAA GCA GTT ACA AGG AGT GAA ATG CTG GCA GTA GTT	940
AAC AAG AAG TAT AAG GAG CAA TTC CCT GAG ATC CTC AGG AGA	982
ACT TCT GCA CGC CTA GAA TTA GTC TTT GGT CTT GAG TTG AAG	1024
GAA ATT GAT CCC AGC ACT CAT TCC TAT TTG CTG GTA GGC AAA	1066
CTG GGT CTT TCC ACT GAG GGA AGT TTG AGT AGT AAC TGG GGG	1108
TTG CCT AGG ACA GGT CTC CTA ATG TCT GTC CTA GGT GTG ATC	1150
TTC ATG AAG GGT AAC CGT GCC ACT GAG CAA GAG GTC TGG CAA	1192
TTT CTG CAT GGA GTG GGG GTA TAT GCT GGG AAG AAG CAC TTG	1234
ATC TTT GGC GAG CCT GAG GAG TTT ATA AGA GAT GTA GTG CGG	1276
GAA AAT TAC CTG GAG TAC CGC CAG GTA CCT GGC AGT GAT CCC	1314
CCA AGC TAT GAG TTC CTG TGG GGA CCC AGA GCC CAT GCT GAA	1360
ACA ACC AAG ATG AAA GTC CTG GAA GTT TTA GCT AAA GTC AAT	1402
GGC ACA GTC CCT AGT GCC TTC CCT AAT CTC TAC CAG TTG GCT	1444
CTT AGA GAT CAG GCA GGA GGG GTG CCA AGA AGG AGA GTT CAA	1486
GGC AAG GGT GTT CAT TCC AAG GCC CCA TCC CAA AAG TCC TCT	1528
AAC ATG TAG	1537
TTGAGTCTGT TCTGTTGTGT TTGAAAAACA GTCAGGCTCC TAATCAGTAG	1587
AGAGTTCATA GCCTACCAGA ACCAACATGC ATCCATTCTT GGCCTGTTAT	1637
ACATTAGTAG AATGGAGGCT ATTTTGTGTTA CTTTTCAAAT GTTTGTTTAA	1687
CTAAACAGTG CTTTTTGCCA TGCTTCTTGT TAACTGCATA AAGAGGTAAC	1737
TGTCACCTGT CAGATTAGGA CTGTTTTTGT TATTTGCAAC AAAGTGGAAA	1787
ACATTATTTT GTTTTTACTA AAACATTGTG TAACATTGCA TTGGAGAAGG	1837
GATTGTCTATG GCAATGTGAT ATCATAACAGT GGTGAAACAA CAGTGAAGTG	1887
GGAAAGTTTA TATTGTTAAT TTTGAAAATT TTATGAGTGT GATTGCTGTA	1937
TACTTTTTTC TTTTTTGTAT AATGCTAAGT GAAATAAAGT TGGATTTGAT	1987
GACTTTACTC AAATTCATTA GAAAGTAAAT CGTAAAACTC TATTACTTTA	2037
TTATTTTCTT CAATTATGAA TTAAGCATTG GTTATCTGGA AGTTTCTCCA	2087
GTAGCACAGG ATCTAGTATG AAATGTATCT AGTATAGGCA CTGACAGTGA	2137
GTTATCAGAG TCT	2150

(2) INFORMATION FOR SEQUENCE ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2099 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: smage-II

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

ACCTTATTGG GTCTGTCTGC ATATGCCTCC ACTTGTGTGT AGCAGTCTCA	50
AATGGATCTC TCTCTACAGA CCTCTGTCTG TGTCTGGCAC CCTAAGTGGC	100
TTTGCATGGG CACAGGTTTC TGCCCTGCA TGGAGCTTAA ATAGATCTTT	150
CTCCACAGGC CTATACCCCT GCATTGTAAG TTTAAGTGGC TTTATGTGGA	200
TACAGGTCTC TGCCCTGTA TGCAGGCCTA AGTTTTCTG TCTGCTTAGC	250
CCCTCCAAGT GAAGCTAGTG AAAGATCTAA CCCACTTTTG GAAGTCTGAA	300
ACTAGACTTT TATGCAGTGG CCTAACAAGT TTTAATTTCT TCCACAGGGT	350
TTGCAGAAAA GAGCTTGATC CACGAGTTCG GAAGTCCTGG TATGTTCTTA	400
GAAAGATGTT CTCCTGGAAA GCTTCAAAAG CCAGGTCTCC ATTAAGTCCA	450
AGGTATTCTC TACCTGGTAG TACAGAGGTA CTTACAGGTT GTCATTCTTA	500
TCTTTCCAGA TTCCTGCTG CCAGCTCTTT TACTTCAGCC CTGAGCACAG	550
TCAACATGCC TAGGGGTCAA AAGAGTAAGA CCCGCTCCCG TGCAAAACGA	600
CAGCAGTCAC GCAGGGAGGT TCCAGTAGTT CAGCCCACTG CAGAGGAAGC	650
AGGGTCTTCT CTTGTTGACC AGAGTCTGG GTCCAGCTTC CCTGGTGGTT	700
CTGCTCCTCA GGGTGTGAAA ACCCCTGGAT CTTTTGTTGC AGGTGTATCC	750
TGCACAGGCT CTGGTATAGG TGGTAGAAAT GCTGCTGTCC TGCCTGATAC	800
AAAAAGTTCA GATGGCACCC AGGCAGGGAC TTCCATTAG CACACACTGA	850
AAGATCCTAT CATGAGGAAG GCTAGTGTGC TGATAGAATT CCTGCTAGAT	900

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AAGTTTAAAG	TGAAAGAAGC	AGTTACAAGG	AGTGAAATGC	TGGCAGTAGT	950
TAACAAGAAG	TATAAGGAGC	AATTCCTGA	GATCCTCAGG	AGAACTTCTG	1000
CACGCCTAGA	ATTAGTCTTT	GGTCTTGAGT	TGAAGGAAAT	TGATCCCAGC	1050
ACTCATTCCCT	ATTTGCTGGT	AGGCAAACCTG	GGTCTTTCCA	CTGAGGGAAG	1100
TTTGAGTAGT	AACTGGGGGT	TGCCTAGGAC	AGGTCTCCTA	ATGTCTGTCC	1150
TAGGTGTGAT	CTTCATGAAG	GGTAACCGTG	CCACTGAGCA	AGAGGTCTGG	1200
CAATTTCTGC	ATGGAGTGGG	GGTATATGCT	GGGAAGAAGC	ACTTGATCTT	1250
TGGCGAGCCT	GAGGAGTTTA	TAAGAGATGT	AGTGCGGGAA	AATTACCTGG	1300
AGTACCGCCA	GGTACCTGGC	AGTGATCCCC	CAAGCTATGA	GTTCTGTGG	1350
GGACCCAGAG	CCCATGCTGA	AACAACCAAG	ATGAAAGTCC	TGGAAGTTTT	1400
AGCTAAAGTC	AATGGCACAG	TCCCTAGTGC	CTTCCCTAAT	CTCTACCACT	1450
TGGCTCTTAG	AGATCAGGCA	GGAGGGGTGC	CAAGAAGGAG	AGTTCAAGGC	1500
AAGGGTGTTT	ATTCCAAGGC	CCCATCCCCA	AAGTCCTCTA	ACATGTAGTT	1550
GAGTCTGTTT	TGTTGTGTTT	GAAAAACAGT	CAGGCTCCTA	ATCAGTAGAG	1600
AGTTCATAGC	CTACCGAAGC	CAACATGCAT	CCATTCTTGG	CCTGTTATAC	1650
ATTAGTAGAA	TGGAGGCTAT	TTTTGTTACT	TTTCAAATGT	TGTTTAACT	1700
AAACAGTGCT	TTTTGCCATG	CTTCTTGTTA	ACTGCATAAA	GAGGTAAGTC	1750
TCACTTGTC	GATTAGGACT	TGTTTTGTTA	TTTGCAACAA	ACTGGAAAAC	1800
ATTATTTTGT	TTTTACTAAA	ACATTGTGTA	ACATTGCATT	GGAGAAGGGA	1850
TTGTCATGGC	AATGTGATAT	CATACAGTGG	TGAAACAACA	GTGAAGTGGG	1900
AAAGTTTATA	TTGTTAGTTT	TGAAATTTT	ATGAGTGTGA	TTGCTGTATA	1950
CTTTTTTCTT	TTTTGTATAA	TGCTAAGTGA	AATAAAGTTG	GATTTGATGA	2000
CTTTACTCAA	ATTATTAGA	AAGTAAATCA	TAAAACTCTA	TTACTTTATT	2050
ATTTTCTTCA	ATTATTAATT	AAGCATTGGT	TATCTGGAAG	TTTCTCCAG	2099

- (2) INFORMATION FOR SEQUENCE ID NO: 26:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acids
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Glu Ala Asp Pro Thr Gly His Ser Tyr
5

- (2) INFORMATION FOR SEQUENCE ID NO: 27:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

TGGAGGACCA GAGGCCCCC

19

- (2) INFORMATION FOR SEQUENCE ID NO: 28:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GGACGATTAT CAGGAGGCCT GC

22

87

- (2) INFORMATION FOR SEQUENCE ID NO: 29:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GAGCAGACAG GCCAACCG

18

- (2) INFORMATION FOR SEQUENCE ID NO: 30:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

AAGGACTCTG CGTCAGGC

18

- (2) INFORMATION FOR SEQUENCE ID NO: 31:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CTAGAGGAGC ACCAAAGGAG AAG

23

- (2) INFORMATION FOR SEQUENCE ID NO: 32:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

TGCTCGGAAC ACAGACTCTG G

21

- (2) INFORMATION FOR SEQUENCE ID NO: 33:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

TGGAGGACCA GAGGCCCCC

19

88

- (2) INFORMATION FOR SEQUENCE ID NO: 34:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CAGGATGATT ATCAGGAAGC CTGT

24

- (2) INFORMATION FOR SEQUENCE ID NO: 35:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CAGAGGAGCA CCGAAGGAGA A

21

- (2) INFORMATION FOR SEQUENCE ID NO: 36:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

CAGGTGAGCG GGGTGTGTC

19

- (2) INFORMATION FOR SEQUENCE ID NO: 37:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

CCCCAGAGAA GCACTGAAGA AG

22

- (2) INFORMATION FOR SEQUENCE ID NO: 38:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GGTGAGCTGG GTCCGGG

17

89

- (2) INFORMATION FOR SEQUENCE ID NO: 39:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CCCCAGAGCA GCACTGACG

19

- (2) INFORMATION FOR SEQUENCE ID NO: 40:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

CAGCTGAGCT GGGTCGACC

19

- (2) INFORMATION FOR SEQUENCE ID NO: 41:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

CACAGAGCAG CACTGAAGGA G

21

- (2) INFORMATION FOR SEQUENCE ID NO: 42:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

CTGGGTAAAG ACTCACTGTC TGG

23

- (2) INFORMATION FOR SEQUENCE ID NO: 43:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GAGAACCCAG AGGATCACTG GA

22

90

- (2) INFORMATION FOR SEQUENCE ID NO: 44:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GGGAAAAGGA CTCAGGGTCT ATC

23

- (2) INFORMATION FOR SEQUENCE ID NO: 45:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GGTGGAAGTG GTCCGCATCG

20

- (2) INFORMATION FOR SEQUENCE ID NO: 46:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

GCCCTCCACT GATCTTTAGC AA

22

- (2) INFORMATION FOR SEQ ID NO: 47:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

CGGCCGAAGG AACCTGACCC AG

22

- (2) INFORMATION FOR SEQ ID NO: 48:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

GCTGGAACCC TCACTGGGTT GCC

23

91

- (2) INFORMATION FOR SEQ ID NO: 49:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

AAGTAGGACC CGAGGCACTG

20

- (2) INFORMATION FOR SEQ ID NO: 50:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

GAAGAGGAAG AAGCGGTCTG

20

- (2) INFORMATION FOR SEQ ID NO: 51:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

TGGAGGACCA GAGGCCCCC

19

- (2) INFORMATION FOR SEQ ID NO: 52:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

GGACGATTAT CAGGAGGCCT GC

22

- (2) INFORMATION FOR SEQ ID NO: 53:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

ACTCAGCTCC TCCCAGATT

20

92

- (2) INFORMATION FOR SEQ ID NO: 54:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

GAAGAGGAGG GGCCAAG

17

- (2) INFORMATION FOR SEQ ID NO: 55:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

TCTTGATATCC TGGAGTCC

18

- (2) INFORMATION FOR SEQ ID NO: 56:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

TTGCCAAGAT CTCAGGAA

18

Claims:

1. Isolated nucleic acid molecule useful as a primer in specifically determining expression of a member of the MAGE family of tumor rejection antigen precursor.
2. The isolated nucleic acid molecule of claim 1, selected from the group consisting of one of SEQ ID NOS: 27-48.
3. A kit useful in determining expression of a MAGE tumor rejection antigen precursor, comprising at least one part of SEQ ID NOS: 27 and 28, 29 and 30, 31 and 32, 33 and 34, 35 and 36, 37 and 38, 39 and 40, 41 and 42, 43 and 44, 45 and 46, and 47-48.
4. Method for determining expression of a MAGE tumor rejection antigen precursor in a cell comprising contacting said cell sample with at least one of the nucleic acid molecules of claim 2 and determining hybridization of said nucleic acid molecule to a target as a determination of expression of MAGE tumor rejection antigen precursor.
5. Method of claim 3, wherein said expression of said tumor rejection antigen precursor is a determination of presence, regression of spread of cancer.
6. The method of claim 5, wherein said cancer is melanoma.
7. The method of claim 5, wherein said cancer is lung adenocarcinoma, said method comprising contacting said sample with a pair of: SEQ ID NOS: 27 and 28, SEQ ID NOS: 47 and 48, or SEQ ID NOS: 49 and 50.

8. The method of claim 4, wherein said cancer is a head squamous cell carcinoma, a neck squamous cell carcinoma, a prostate carcinoma, and a bladder tumor, the method comprising contacting sample with SEQ ID NOS: 27 AND 28, SEQ ID NOS: 29 and 30, SEQ ID NOS: 47 and 48, or SEQ ID NOS: 49 and 50.
9. The method of claim 4, wherein said cancer is a bladder tumor, the method comprising contacting said sample with SEQ ID NOS: 47 and 48, SEQ ID NOS: 49 and 50, or SEQ ID NOS: 51 and 52, followed by amplification.
10. The method of claim 4, wherein said MAGE tumor rejection antigen precursor is MAGE-1, MAGE-2, MAGE-3 or MAGE-4.

AMENDED CLAIMS

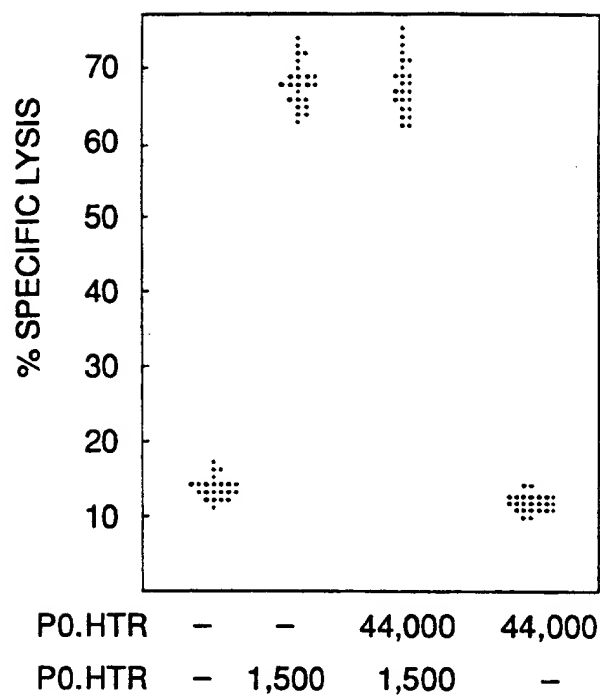
[received by the International Bureau on 27 June 1995 (27.06.95);
original claims 1-10 replaced by amended claims 1,9 (2 pages)]

1. Isolated nucleic acid molecule useful as a primer in specifically determining expression of a member of the MAGE group of tumor rejection antigen precursors, selected from the group consisting of SEQ ID NOS: 27-46.
2. Kit useful in determining expression of a MAGE tumor rejection antigen precursor, comprising at least one pair of:
 - SEQ ID NOS: 27 and 28
 - SEQ ID NOS: 29 and 30
 - SEQ ID NOS: 31 and 32
 - SEQ ID NOS: 33 and 34
 - SEQ ID NOS: 35 and 36
 - SEQ ID NOS: 37 and 38
 - SEQ ID NOS: 39 and 40
 - SEQ ID NOS: 41 and 42
 - SEQ ID NOS: 43 and 44
 - SEQ ID NOS: 45 and 46.
3. Method for determining expression of a MAGE tumor rejection antigen precursor in a cell comprising contacting said cell sample with at least one isolated nucleic acid molecule of claim 1 and determining hybridization of said nucleic acid molecule to a target as a determination of expression of MAGE tumor rejection antigen precursor.
4. The method of claim 3, wherein said expression of said tumor rejection antigen precursor is a determination of presence, regression of spread of cancer.
5. The method of claim 4, wherein said cancer is melanoma.

6. The method of claim 4, comprising contacting said sample with SEQ ID NOS: 27 and 28.
7. The method of claim 4, wherein said cancer is a head squamous cell carcinoma, a neck squamous cell carcinoma, a prostate carcinoma and a bladder tumor, the method comprising contacting said sample with SEQ ID NOS: 27 and 28 or SEQ ID NOS: 29 and 30.
8. The method of claim 4, wherein said cancer is a bladder tumor, said method comprising contacting said sample with SEQ ID NOS: 51 and 52, followed by amplification.
9. The method of claim 4, wherein said MAGE tumor rejection antigen precursor is MAGE-1, MAGE-2, MAGE-3 or MAGE-4.

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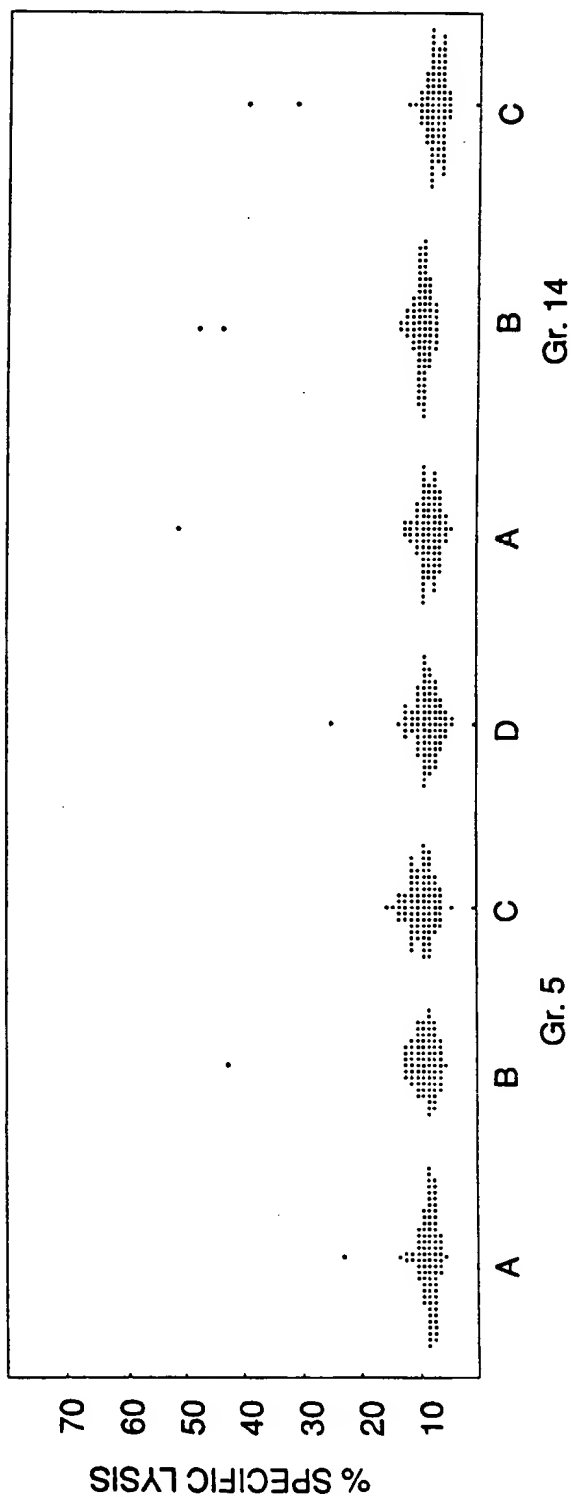
FIG. 1A



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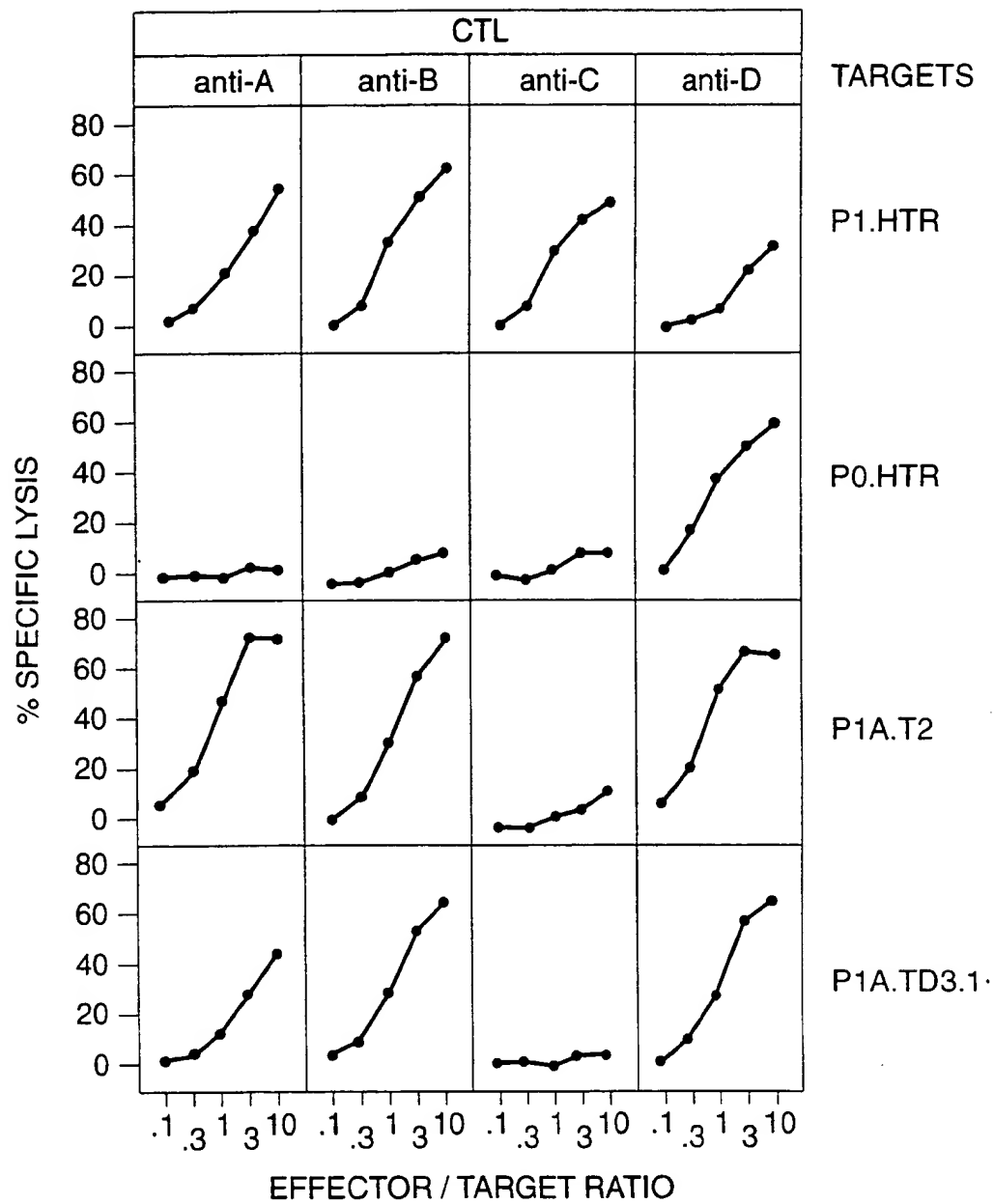
FIG. 1B



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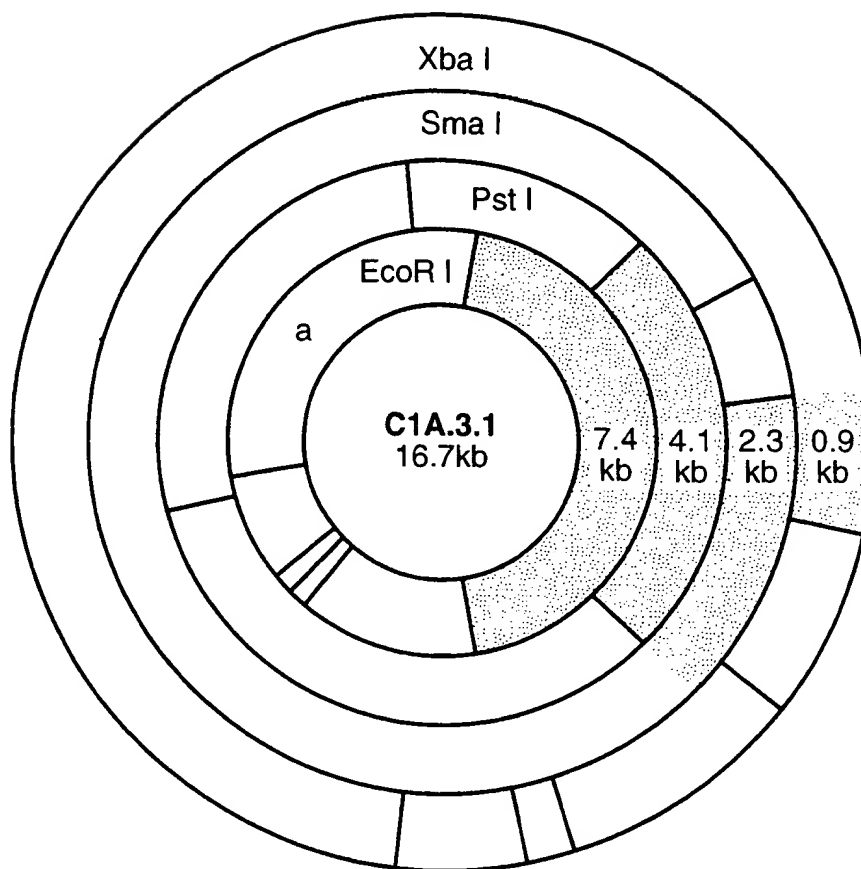
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FIG. 2



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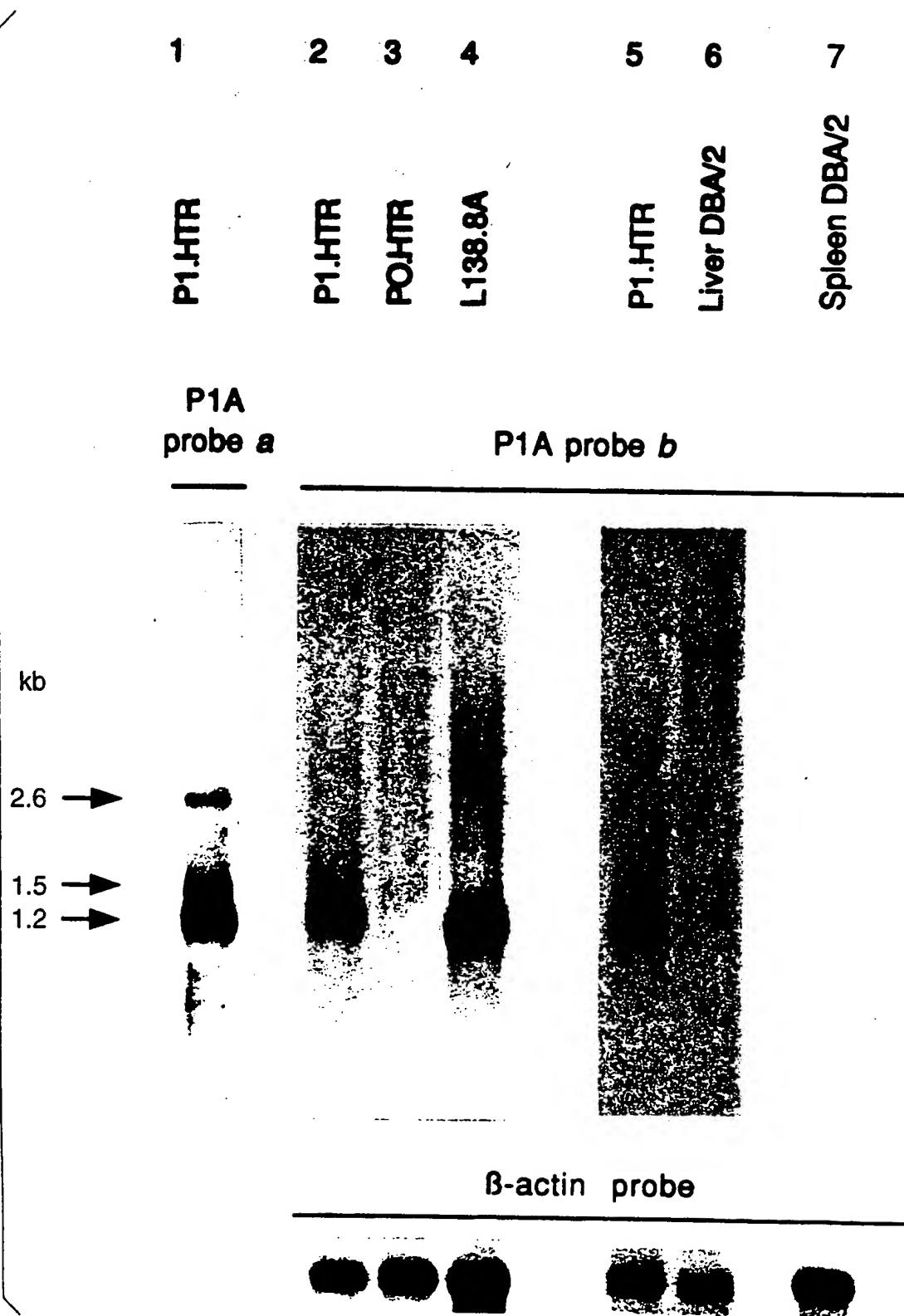
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FIG. 3

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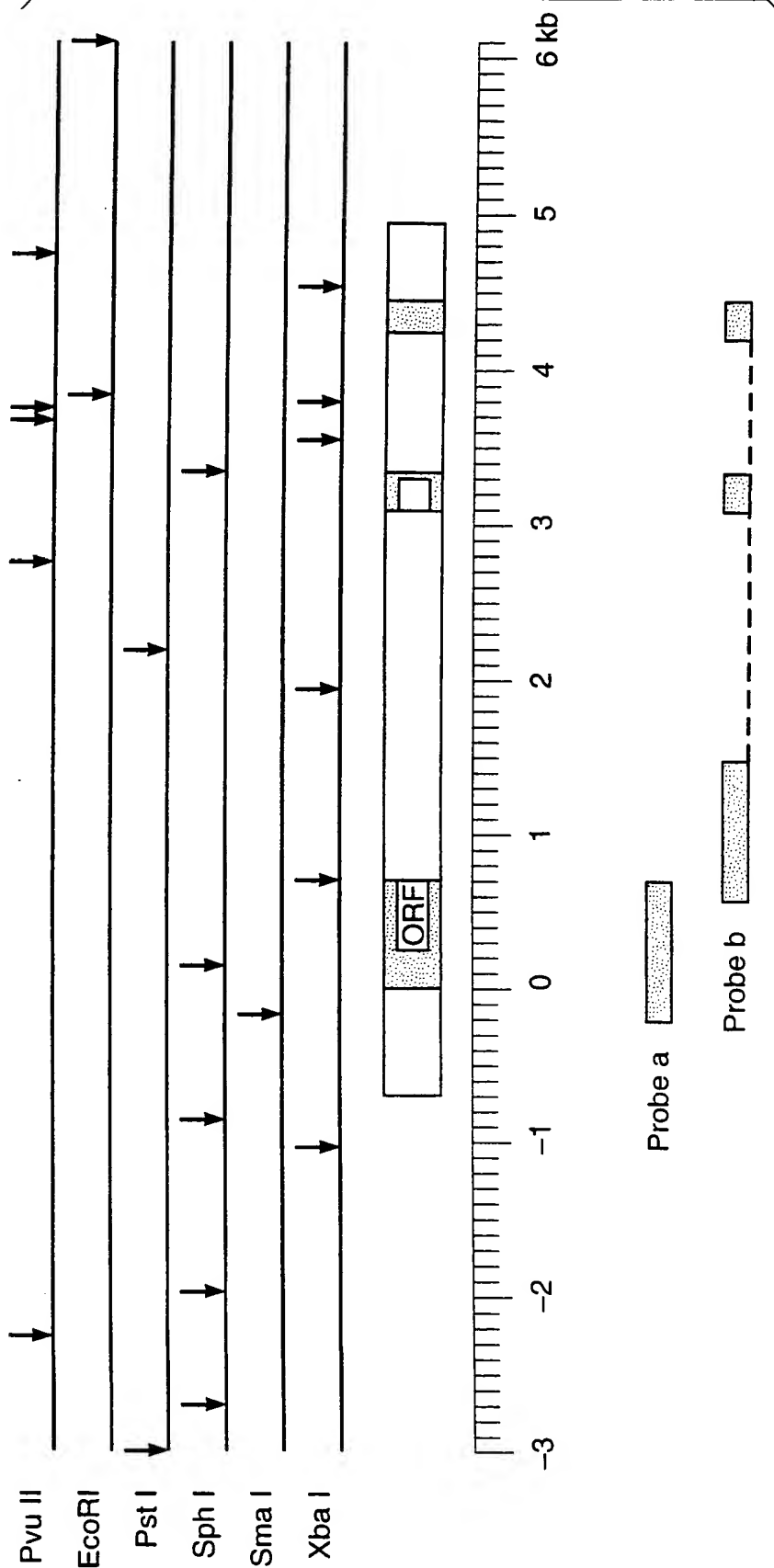
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FIG. 4



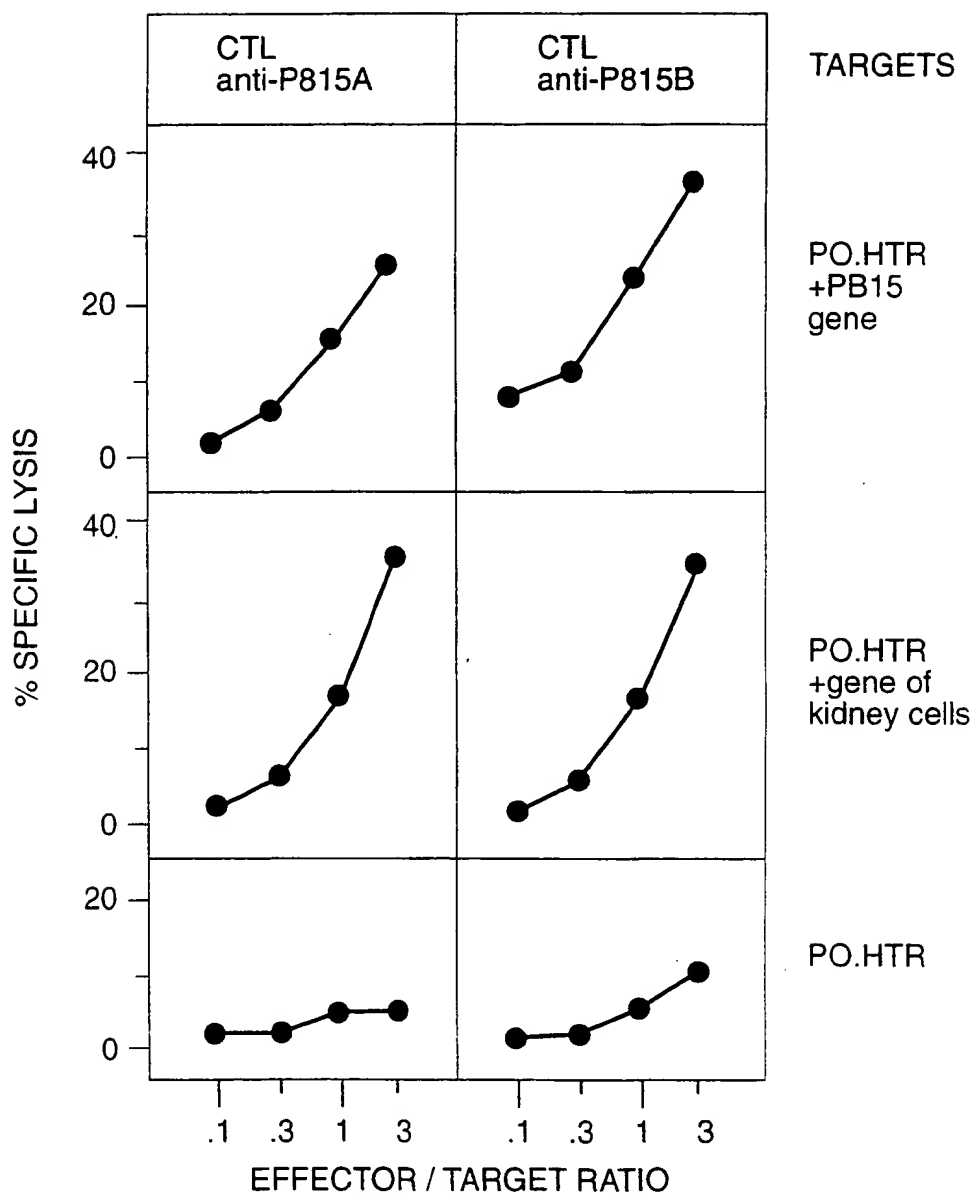
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FIG. 5



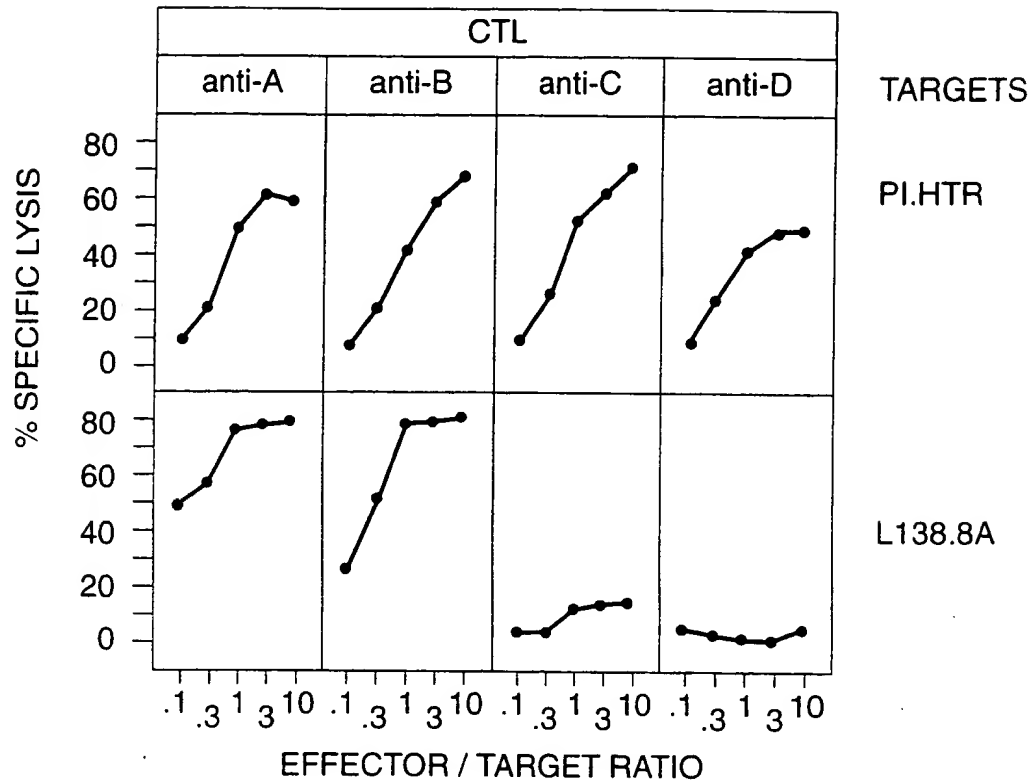
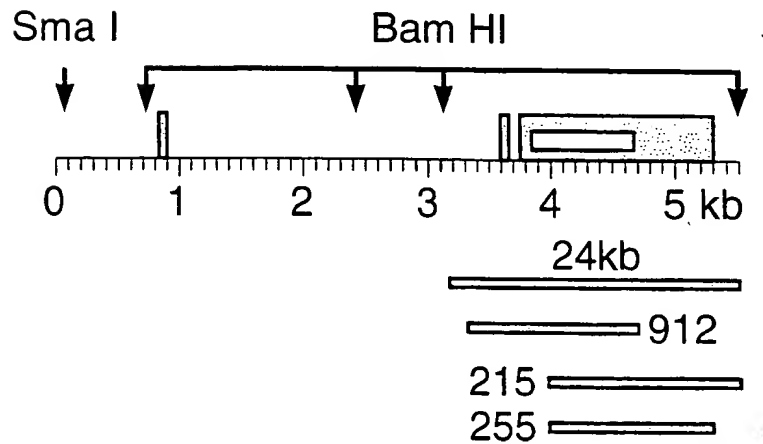
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FIG. 6



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FIG. 7**FIG. 8**

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Fig. 9

MAGE-3 III CCTCCCCAGAGTCTCAGGGAGCCCTCGagCCTcCCCCACTACCATgAACTaCCTcTCTcTgGAGcCAATCCtLaTGAGGacTCCAGCAaCCaaGAAAGAGGAGG
MAGE-2 II CCTCCCCCaCaAGTCTCTCAGGGAGCCCTCCagCTTtCtCgACTACCATCAACtTaCACTCTtTgGAGaCAATCCgaTTGAGGGcTCCAGCAaCCaaGAAAGAGGAGG
MAGE-1 I CCTCCCCAGAGTCTCAGGGAGCCCTCGCCtTTTCCCACTACCAATCAACtTtCACTCGACAGAGGCAACCCAGTGAGGGTtTCCAGGAGCCGTGAAGAGGAGG
225 CHO-8

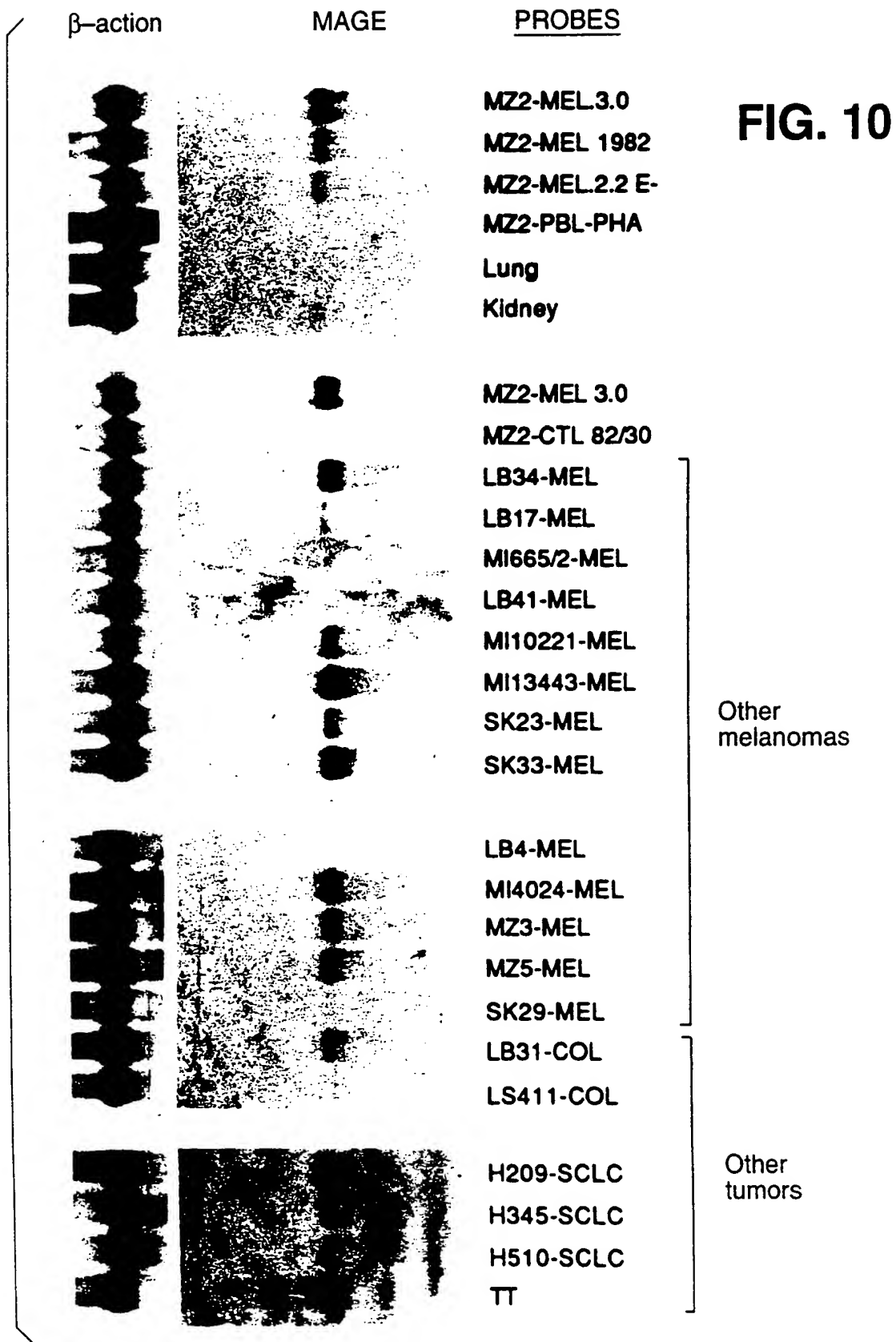
III GGCCAAAGCACCtTcccTgaCC-TGGAGTCCgaGTTCCaAGCAGcAcTCAgTAgGAAGGTGGCCgAGTTGGTtCaTTTtCTGCTCTCTCAAgTATTCGAGGCCA
II GGCCAAAGaAtgTtTtcccgaCCtTGGAGTCCGAGTtTCCaAGCAGcAAATCAgTAgCAAGaTGGTtTGAGTtGGTtTtCaTTTtCTGCTCTCTCAAgTATTCGAGGCCA
I GGCCAAAGCAcCTCTtGTATCC-TGGAGTCCtTTGTtTCCGAGCAGTAATCACTAAAGAGGTGGCTGATtTTGGTtTtTtCTGCTCTCTCAAAATATTCGAGGCCA
325

III GGGAGCCGgtTCACAAAGGCAGAAATGCTTGGG-CAGTGTCTgTcGgAAATtTgCgAGtATtCtTtTtTCTCTGcGATCTTCaGCAAAAGCCTTCcagTTCCTTTCAGCT
II GGGAGCCGgtTCACAAAGGCAGAAATGCTTGGAGAGTGTCTcTCAgAAATtTgCgAGtACTtCtTtTCTCCcGtGATCTTCaGCAAAAGCCTTCcGAGTATtTTCAGCT
I GGGAGCCAGTcACAAAGGCAGAAATGCTTGGAGAGTGTcATCAAAATtTACAGACtGTtTtTtTCTCTGAGATCTTTCGGCAAAAGCCTCTcGAGTCTCTTTCAGCT
425

III GGTCTTTTGGCATcGAgcTGAcGGAAAGtGgGACCCCAcCGGCCACTtGtTAcATCTtTtGcCACCtTgCCCTgGcCCTCTCTACcGATGGCCCTGCTGGGTGACAAAT
II GGTCTTTTGGCATcGAGTGTgTGGAAAGtGgGtCCCCAtCaGCCACTtGtTAcATCTCTCTTGTCACTTGGCCCTGCTGCTGGGTGACAAAT
I GGTCTTTTGGCATtTGCAATtGAGAGCAGACCCACCGGCCACTCTCTATGTCTtTGTCACTTGCCTAGGTCTCTCTCTATGTATGGCCCTGCTGGGTGATTAAT
525

III CAGATCATGCCCAAGgCAGGCCtTCTCTGATAATcGTCTTGGCCATATcGCCAAGaGAGGGCCGaCtGtTGCcCCCTGAGGAGaAAATCTTGGGAGGAGCTTGAGTg
II CAgTTCATGCCCAAGACAGGCCtTCTCTGATAATcGTCT-TGGCCATATcGCAATaGAGGGCCGaCtGtTGCcCCCTGAGGAGaAAATCTTGGGAGGAGCTGAGTg
I CAgTTCATGCCCAAGACAGGCCtTCTCTGATAATtTGTCTCTGCTCATGTATGCAATGGAGGGCCGGCCATGTCTCTTGAGGAGGAAATCTTGGGAGGAGCTGAGTg
625 CHO

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FIG. 11A

FIG. 11A

		EXPRESSION OF MAGE GENE FAMILY				RECOGNITION BY ANI-E CTL		Expression of antigen MZ2-E after transfection**	
		Northern blot probed with cross-reactive MAGE-1 probe*	cDNA-PCR product probed with oligonucleotide specific for:			tested by:			
			MAGE-1	MAGE-2	MAGE-3†	TNF release‡	Lysis§		
Cells of patient MZ2	melanoma cell line MZ2-MEL.3.0	+	+++	+++	+++	+	+		
	tumor sample MZ2 (1982)	+	+++	+++	+++				
	antigen-loss variant MZ2-MEL.2.2	+	-	+++	+++	-	-		
	CTL clone MZ2-CTL.82/30	-	-	-	-				
	PHA-activated blood lymphocytes	-	-	-	-				
Normal tissues	Liver	-	-	-	-				
	Muscle	-	-	-	-				
	Skin	-	-	-	-				
	Lung	-	-	-	-				
	Brain	-	-	-	-				
	Kidney	-	-	-	-				
Melanoma cell lines of HLA-A1 patients	LB34-MEL	+	++	+++	+++	+	+	+	
	MI665/2-MEL	-	-	-	-	-	-	+	
	MI10221-MEL	+	-	++	+++	-	-		
	MI13443-MEL	+	+++	+++	+++	+	+	-	
	SK33-MEL	+	-	+++	+++	-	-		
	SK23-MEL	+	-	+++	+++	-	+	+	

* Data obtained in the conditions of figure 5.

† Data obtained as described in figure 6.

‡ TNF release by CTL 82/30 after stimulation with the tumor cells as described in (11).

§ Lysis of 51 Cr labelled target by CTL 82/30 in the conditions of figure 1.

** Cells transfected with the 2.4 kb fragment of gene MAGE-1 were tested for their ability to stimulate TNF release by CTL 82/30

FIG. 11B

	EXPRESSION OF MAGE GENE FAMILY				RECOGNITION BY ANI-E CTL	
	Northern blot probed with cross-reactive MAGE-1 probe*	cDNA-PCR product probed with oligonucleotide specific for:			tested by: TNF release†	Lysis‡
		MAGE-1	MAGE-2	MAGE-3†		
Melanoma cell lines of other patients						
	+	+	+++	+++	-	-
	+	-	+++	+++	-	-
	-	-	-	-	-	-
	-	-	-	-	-	-
	+	+++	+++	+++	-	-
	-	-	-	-	-	-
	+	+	+++	+++	-	-
	+	-	+++	+++	-	-
	+	-	+++	+++	-	-
Melanoma tumor sample	+	+++	+	+++	-	-
Other tumor cell lines						
	+	-	+++	+++	-	-
	+	-	+++	+++	-	-
	+	-	+++	+++	-	-
	+	+	+++	+++	-	-
	+	-	-	+++	-	-
	+	+++	+++	+++	-	-
	+	+++	+++	+++	-	-
	-	-	-	-	-	-
Other tumor samples						
	-	-	-	-	-	-
	-	-	-	-	-	-

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* Data obtained in the conditions of figure 5.

† Data obtained as described in figure 6.

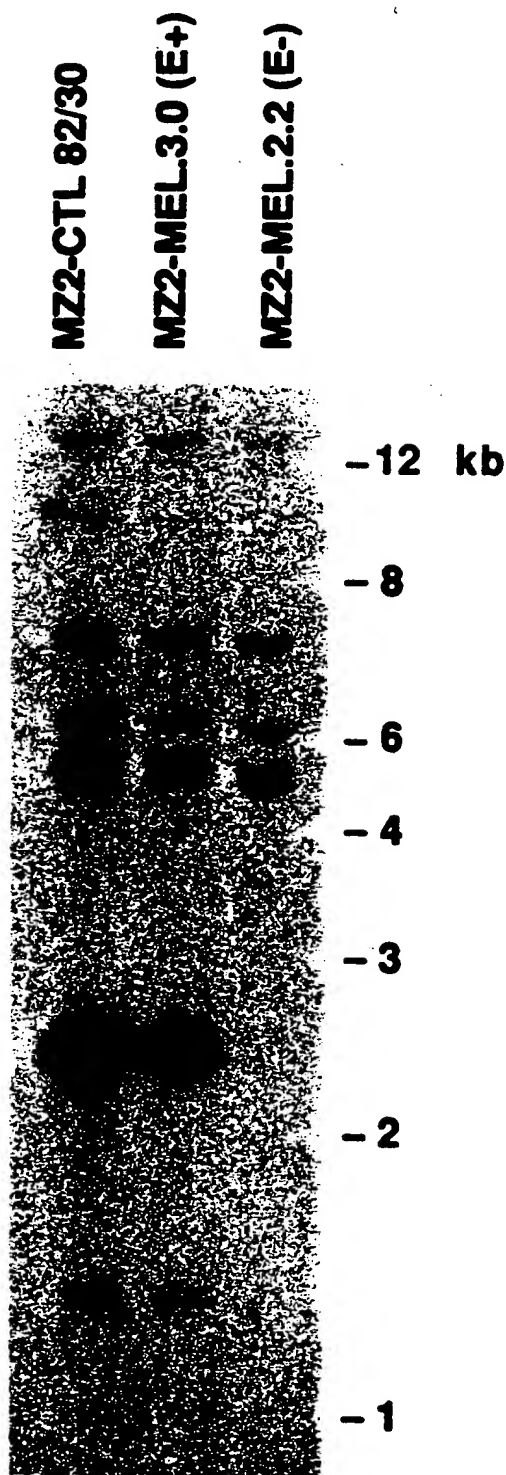
‡ TNF release by CTL 82/30 after stimulation with the tumor cells as described in (11).

§ Lysis of 51 Cr labelled target by CTL 82/30 in the conditions of figure 1.

** Cells transfected with the 2.4 kb fragment of gene MAGE-1 were tested for their ability to stimulate TNF release by CTL 82/30

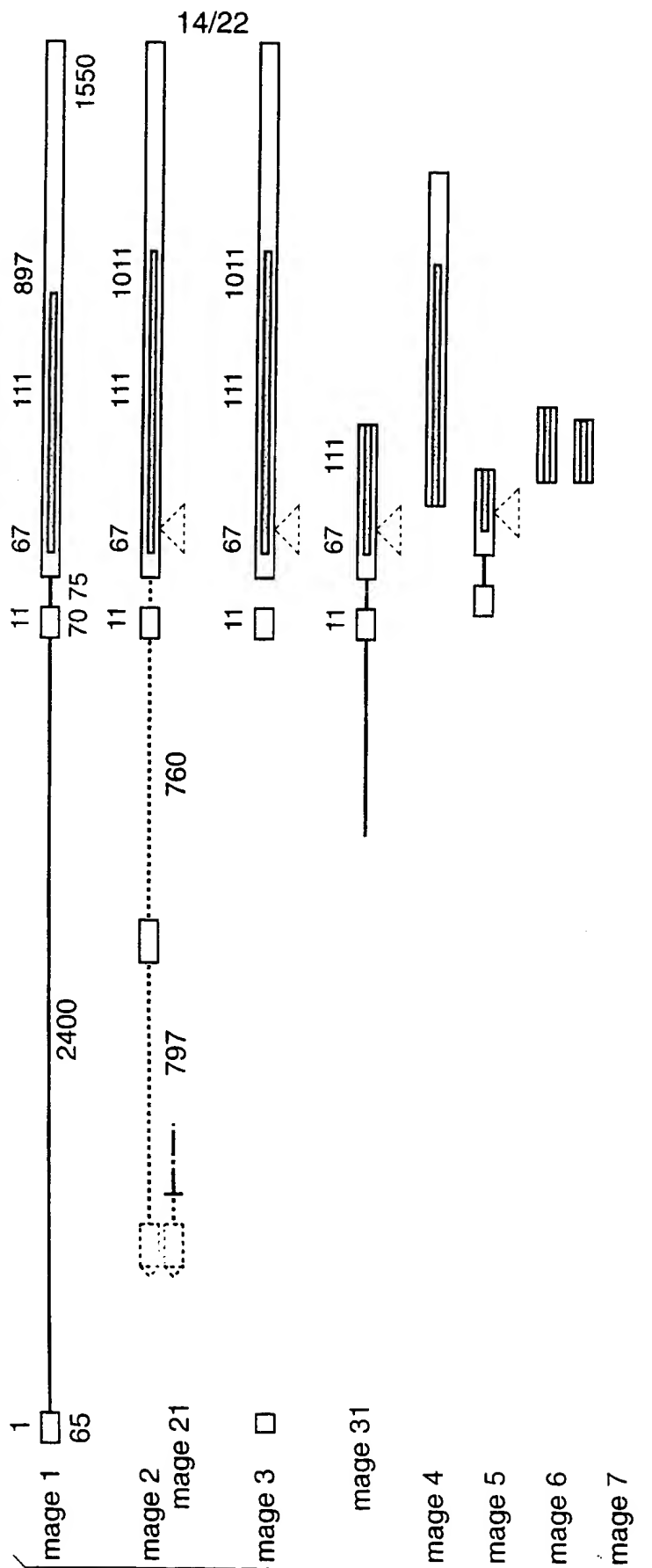
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FIG. 12



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FIG. 13



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FIG. 14B

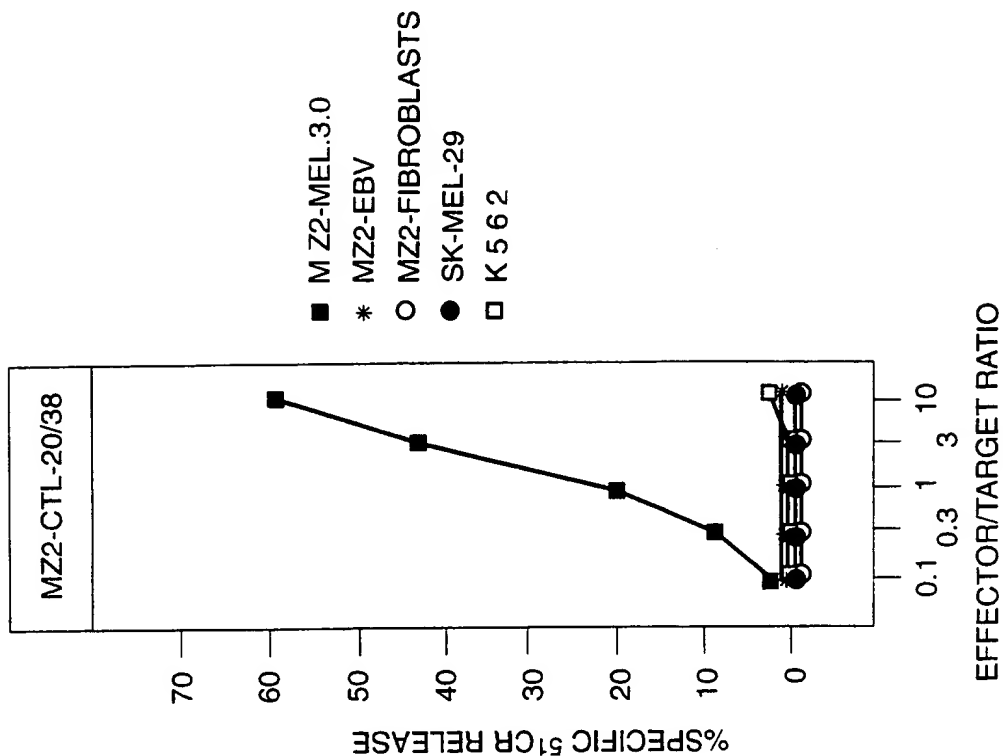
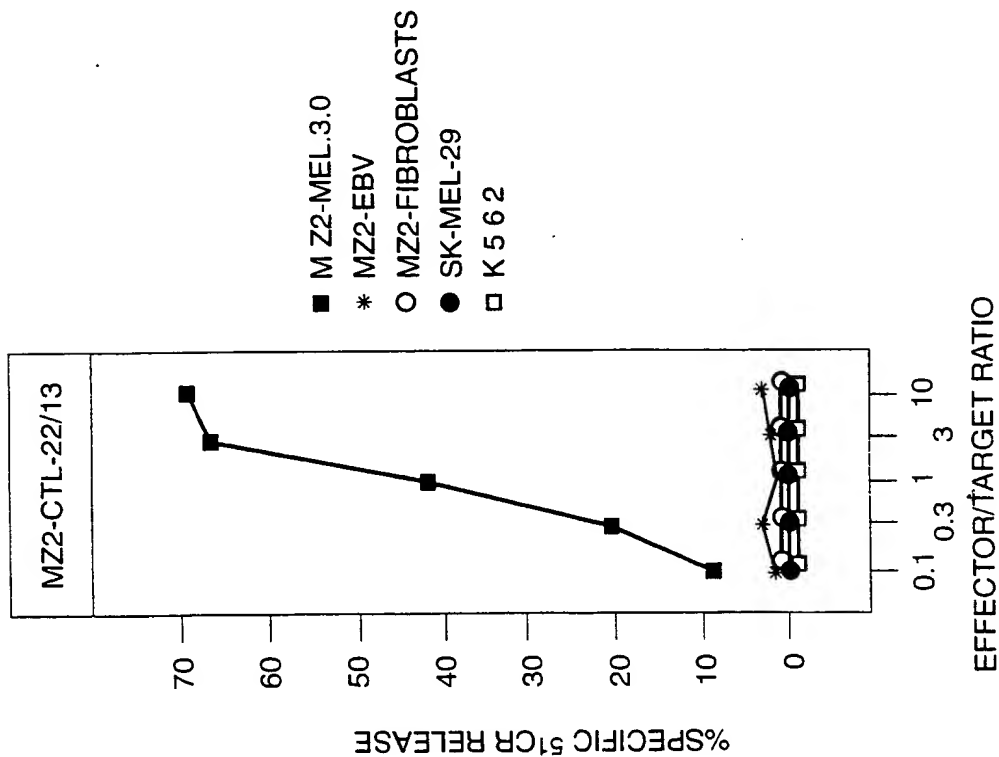
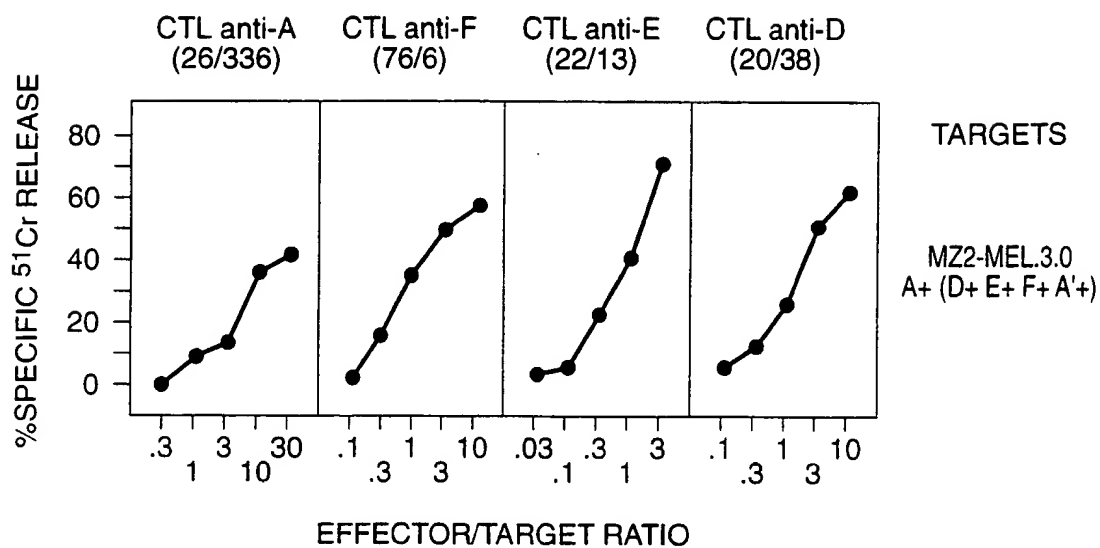
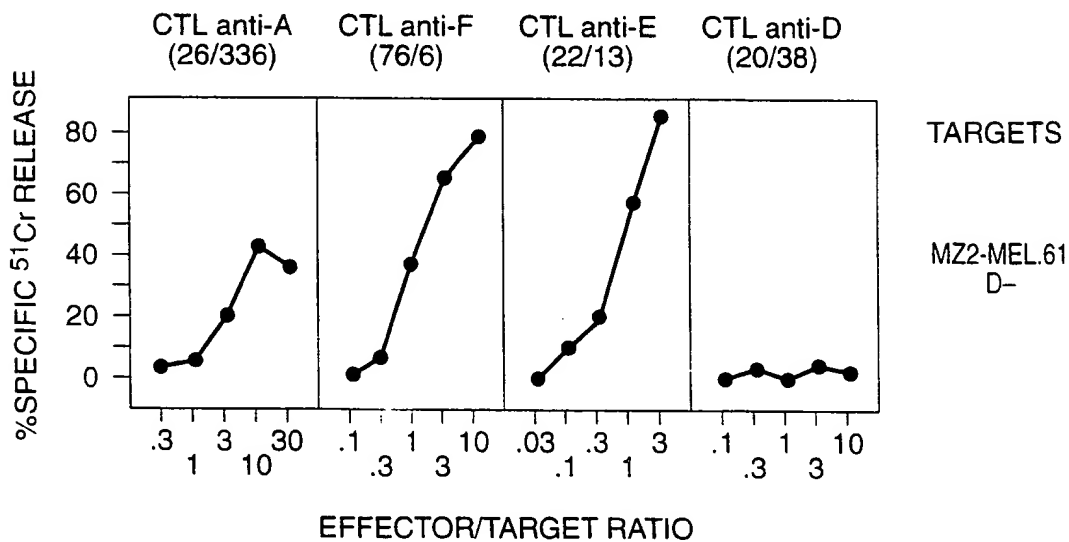


FIG. 14A

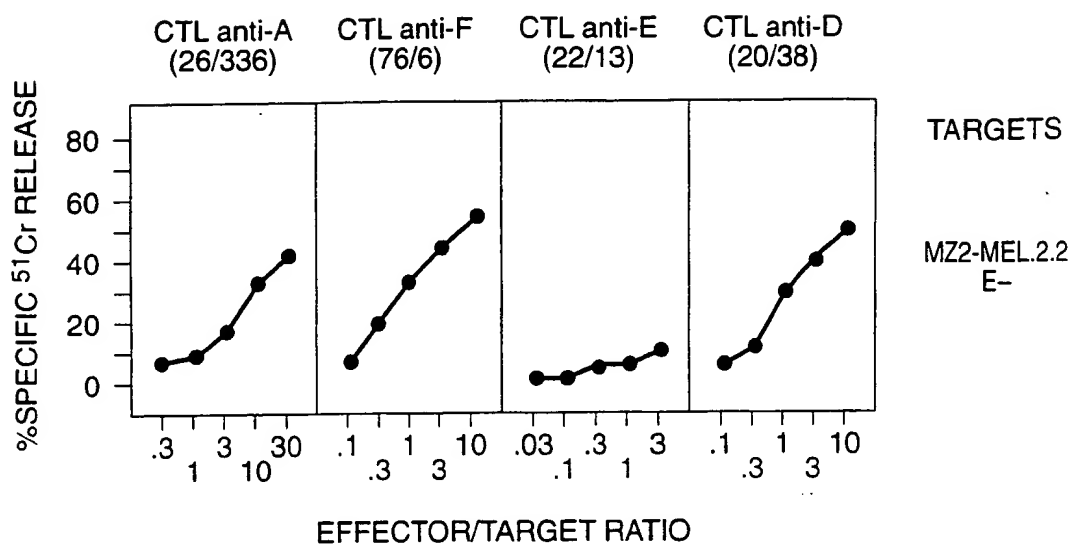
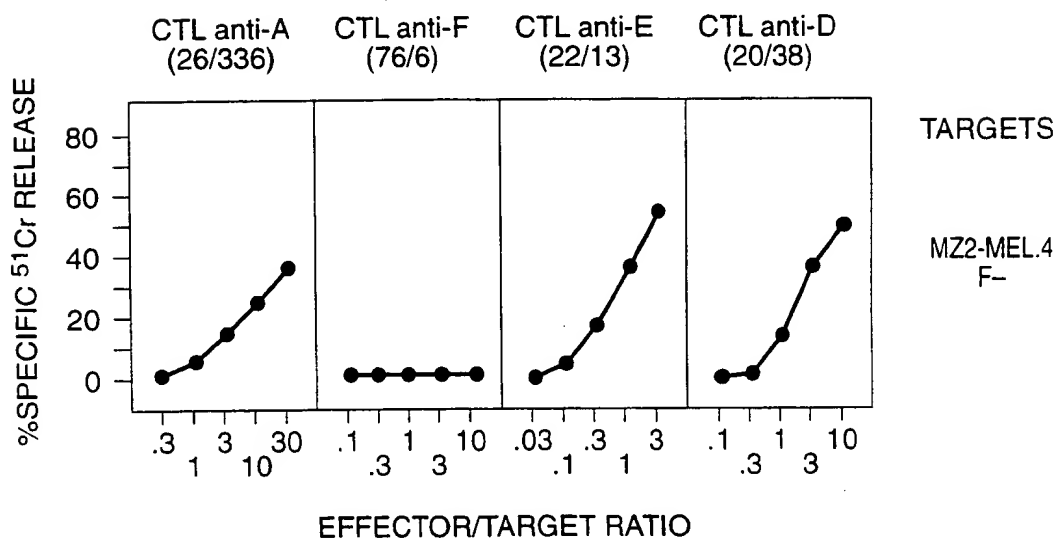


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FIG. 15A**FIG. 15B**

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FIG. 15C**FIG. 15D**

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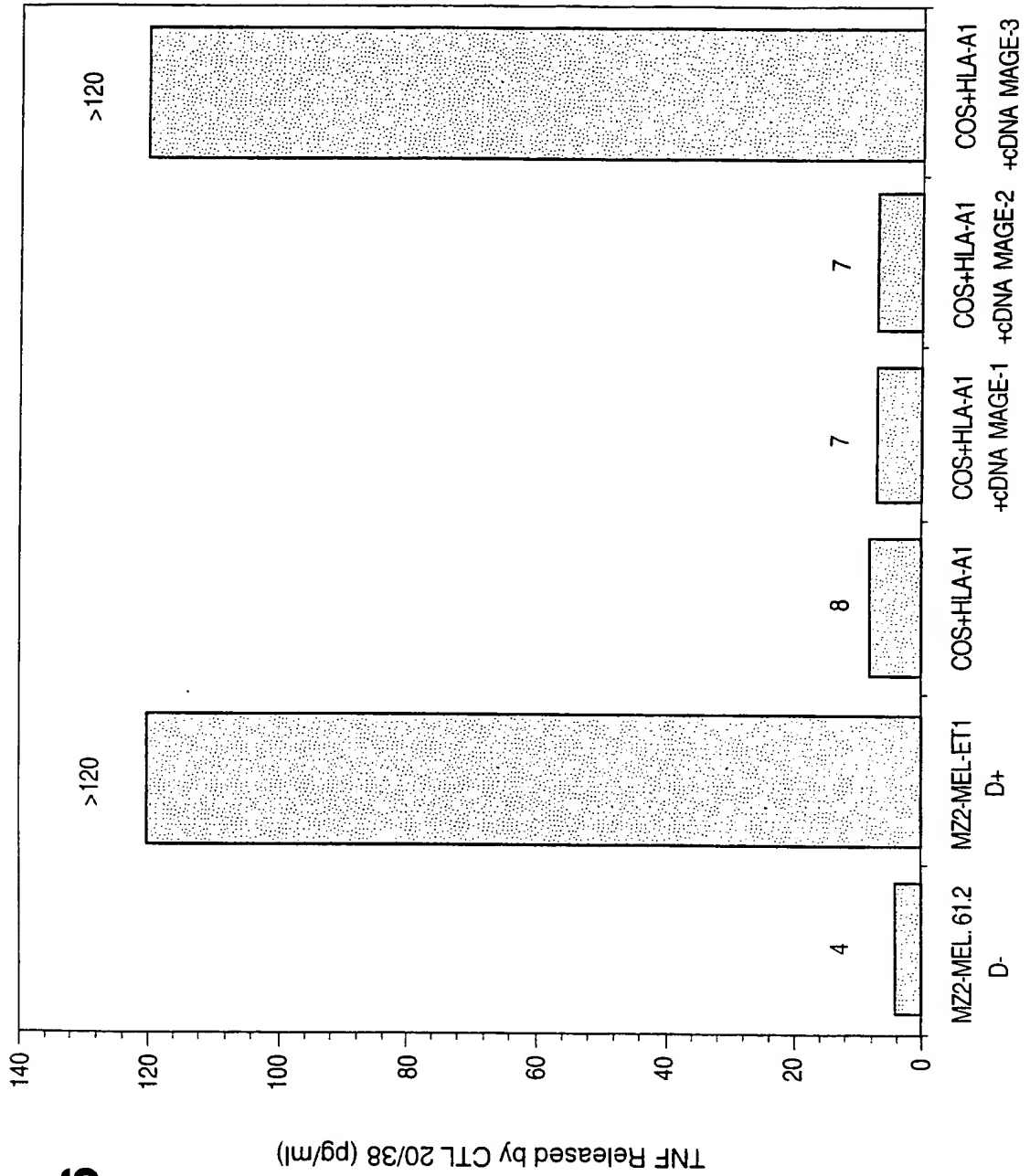
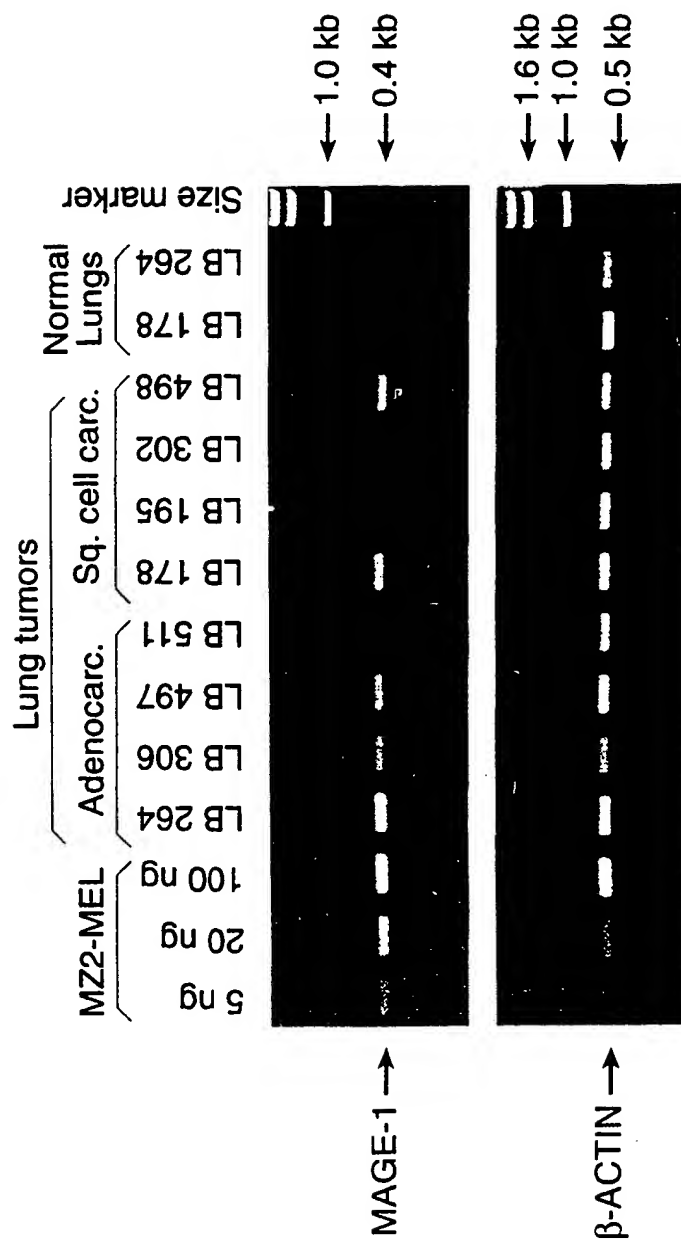


FIG. 16

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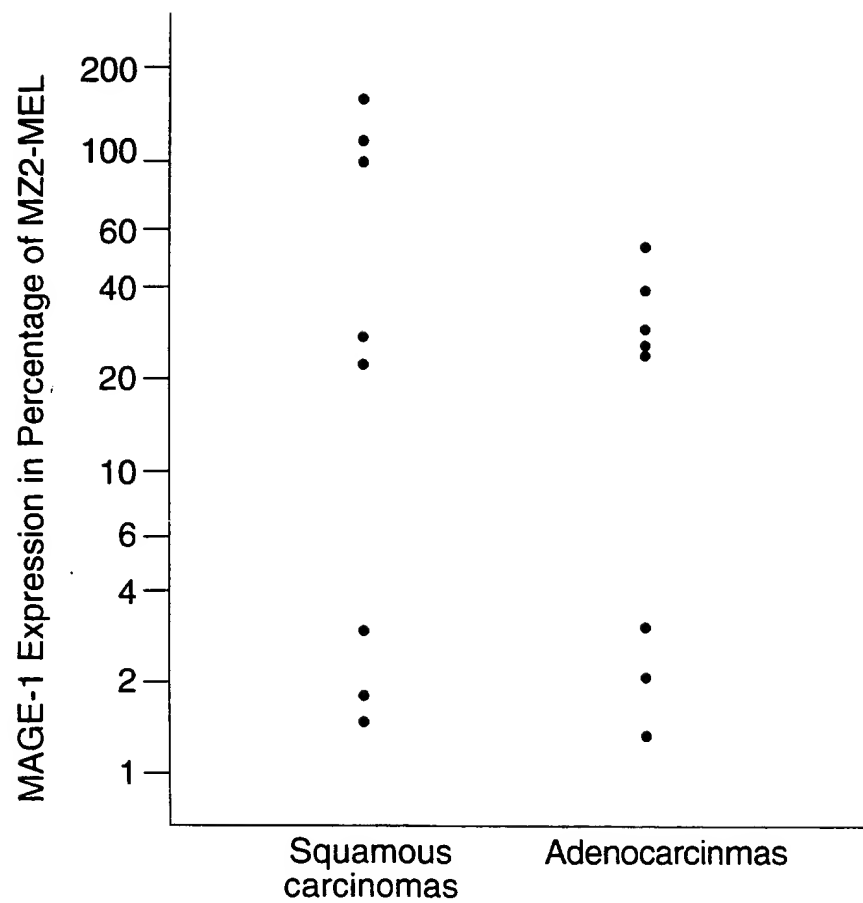
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FIG. 17



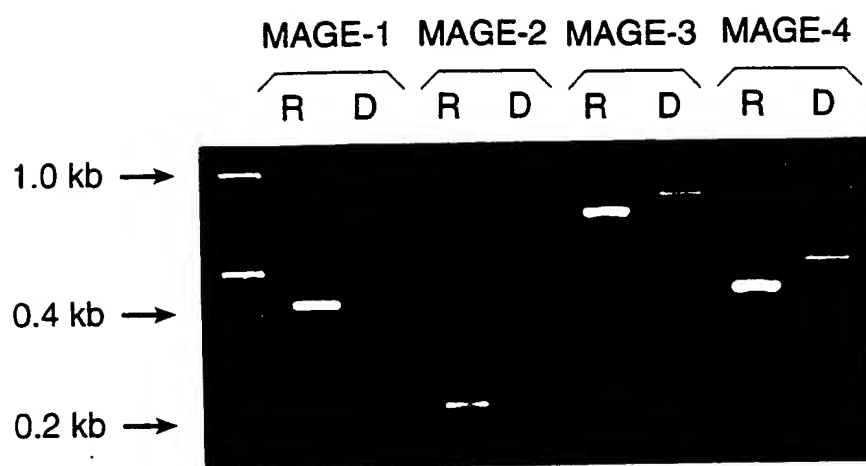
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FIG. 18

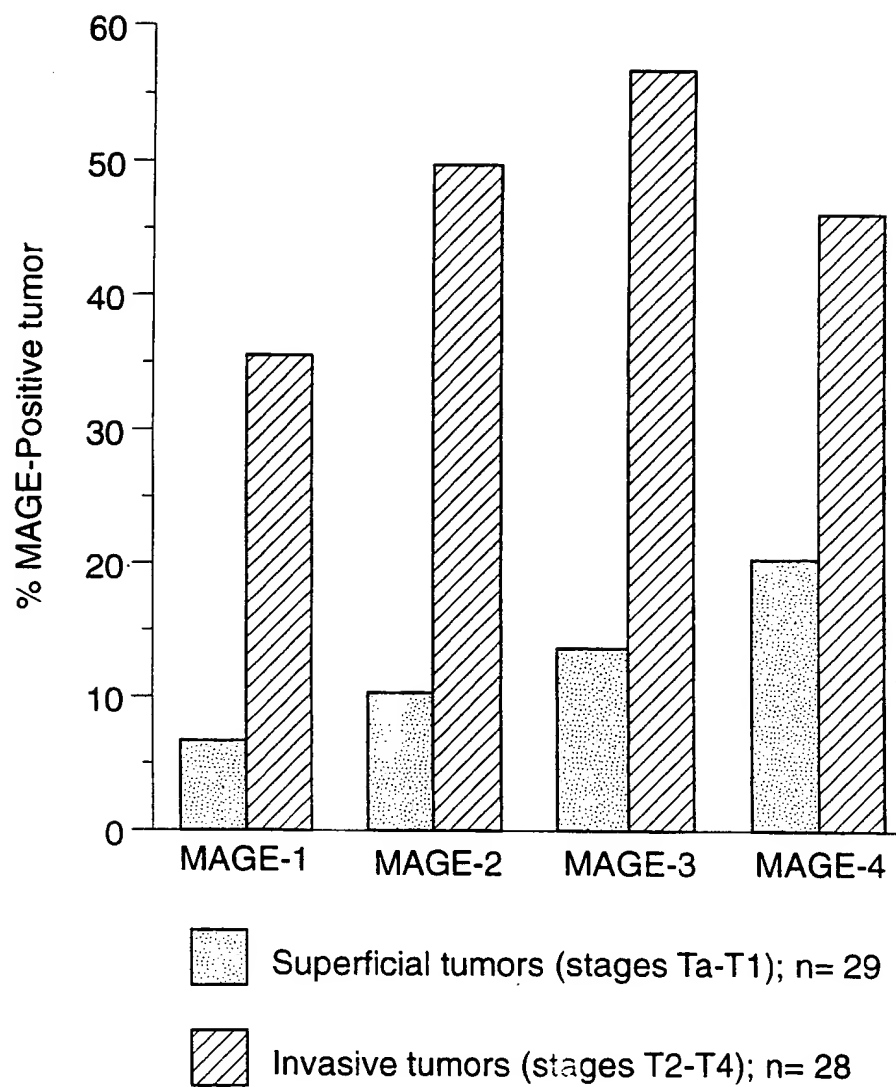
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FIG. 19

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FIG. 20

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02203

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68
US CL : 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, BIOSIS, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	IMMUNOGENETICS, VOLUME 39, ISSUED 1994, SMET ET AL. "SEQUENCE AND EXPRESSION PATTERN OF HUMAN MAGE2 GENE", PAGES 121-129, SEE ENTIRE DOCUMENT.	1-10
Y	INTERNATIONAL JOURNAL OF CANCER, ISSUED 1994, WEYNANTS ET AL, "EXPRESSION OF MAGE GENES BY NON-SMALL-CELL LUNG CARCINOMAS", PAGES 826-829, SEE ENTIRE DOCUMENT.	1-10
Y	WO, A, 92/20356 (BOON ET AL) 26 NOVEMBER 1992, SEE ENTIRE DOCUMENT.	1-10
A,P	US, A, 5,342,774 (BOON ET AL), 30 AUGUST 1994, SEE ENTIRE DOCUMENT.	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

01 MAY 1995

Date of mailing of the international search report

24 MAY 1995

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